Renal Lactate Metabolism and Gluconeogenesis During Insulin-Induced Hypoglycemia

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The contribution of gluconeogenic precursors to renal glucose production (RGP) during insulin-induced hypoglycemia was assessed in conscious dogs. Ten days after surgical placement of sampling catheters in the right and left renal veins and femoral artery and an infusion catheter in the left renal artery, systemic and renal glucose and glycerol kinetics were measured with peripheral infusions of [6-^{3}H]glucose and [2-^{13}C]glycerol. Renal blood flow was determined with a flowprobe, and the renal balance of lactate, alanine, and glycerol was calculated by arteriovenous difference. 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, from 130 ± 8 to 1,825 ± 50 pg/ml; norepinephrine, from 129 ± 6 to 387 ± 15 pg/ml; and glucagon, from 52 ± 2 to 156 ± 12 pg/ml (all P < 0.01). RGP increased from 1.7 ± 0.4 to 3.0 ± 0.5 (left) and from 0.6 ± 0.2 to 3.2 ± 0.2 (right) µmol · kg^{-1} · min^{-1} (P < 0.01). Whole-body glycerol appearance increased from 6.0 ± 0.5 to 7.7 ± 0.7 µmol · kg^{-1} · min^{-1}(P < 0.01); renal conversion of glycerol to glucose increased from 0.13 ± 0.04 to 0.30 ± 0.10 (left) and from 0.11 ± 0.03 to 0.25 ± 0.05 (right) µmol · kg^{-1} · min^{-1}, (P < 0.05). Net renal gluconeogenic precursor uptake increased from 1.5 ± 0.4 to 5.0 ± 0.4 (left) and from 0.9 ± 0.2 to 3.8 ± 0.4 (right) µmol · kg^{-1} · min^{-1} (P < 0.01). Renal lactate uptake could account for ~40% of postabsorptive RGP and for 60% of RGP during hypoglycemia. These results indicate that gluconeogenic precursor extraction by the kidney, particularly lactate, is stimulated by counterregulatory hormones and accounts for a significant fraction of the enhanced gluconeogenesis induced by hypoglycemia. *Diabetes* **47:1101–1106, 1998**

Insulin-induced hypoglycemia triggers the release of various counterregulatory hormones that blunt insulin action by limiting peripheral glucose utilization and increasing glucose production (1). Previous studies have indicated that hepatic glycogenolysis is responsible for the initial increase in glucose production, whereas gluconeogenesis is the primary process by which glucose production is sustained as hypoglycemia is prolonged (2–4). Although these observations underscore the role of the liver in glucose counterregulation, the potential contribution of renal gluconeogenesis to glucose production during insulin-induced hypoglycemia has been largely unappreciated. Estimation of glycogenolysis and gluconeogenesis rates in these studies (2–4) is based on the assumption that the liver is the only organ capable of producing glucose during hypoglycemia. Recent findings, however, have indicated that postabsorptive renal glucose production (RGP) equals glucose utilization and may account for up to ~30% of endogenous glucose production (5), and that glucose production by the kidney, analogous to the liver, is suppressed by insulin (5) and stimulated by catecholamines (6). Not surprisingly, evidence was just reported that glucose released in the renal vein is responsible for a substantial fraction of endogenous glucose appearance in hypoglycemic dogs (7). These observations strongly suggest that renal gluconeogenesis is enhanced and may represent an important component of the body's defense against insulin-induced hypoglycemia.

The ability of mammalian kidney to form glucose from noncarbohydrate precursors was discovered by Benoy and Elliot in 1937 (8) and confirmed by others in subsequent in vivo (9,10) and in vitro (11,12) studies. The metabolic pathways and enzymatic steps of renal gluconeogenesis reflect those found in the liver of the same species; however, several differences between the liver and the kidney regarding substrate specificity are apparent. Whereas lactate, pyruvate, and glycerol are common substrates for both organs, alanine and serine are gluconeogenic only in liver preparations (13,14). On the other hand, only renal cell preparations can avidly transport and convert di- and tricarboxylic acids (malate, α-ketoglutarate, fumarate, succinate, and citrate) to glucose (15). Although in vivo experiments confirm that the hepatic fractional extraction and conversion efficiency of circulating lactate, glycerol, and alanine to glucose is increased (2–4), the potential contribution of these precursors to renal gluconeogenesis during insulin-induced hypoglycemia has not yet been determined. The present study represents an extension of previously published data and was therefore undertaken to examine the role of lactate, glycerol, and alanine utilization by the kidney in gluconeogenesis during insulin-induced hypoglycemia using arteriovenous balance combined with a multiple tracer technique.

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Received for publication 2 December 1997 and accepted in revised form 1 April 1998.

RGP, renal glucose production.

RESEARCH DESIGN AND METHODS

Animals and surgery. After approval by the Institutional Animal Care and Use Committee at the State University of New York at Stony Brook, studies were performed in six 20-to 25-kg male mongrel dogs. Anesthesia, surgical techniques, and catheter sampling were detailed previously (7).

Experimental protocol. After an overnight fast, catheters were exteriorized, and an infusion catheter was inserted into the precava via a lateral saphenous vein. At 8:00 A.M. (t = –120 min), primed constant systemic infusions of [6-^{3}H]glucose

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(10 µCi, 0.20 µCi/min) and [2-^{13}C]glycerol (45 µmol/kg, 0.45 µmol · kg^{-1} · min^{-1}; Cambridge Isotope Laboratories, Andover, MA) were 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 and right and left renal vein blood samples were obtained every 10 min from t = –30 to 0 min for the measurement of plasma glucose concentration and specific activity; [2-^{13}C]glucose and glycerol plasma enrichment; plasma insulin, glucagon, catecholamines, lactate, glycerol, and alanine concentrations; 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}) was 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 unilateral renal plasma flow of 5 ml · kg^{-1} · min^{-1} (5). Blood samples were obtained every 10 min between 90 and 120 min for the same measurements as in the baseline period. In addition, femoral artery and left renal vein blood samples were obtained for the determination of [6,6-^{2}H]glucose plasma enrichment. At the end, each dog was euthanized, 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 specific activity was determined in deproteinized plasma (16) after deionization with ion exchange resins. Plasma insulin (17) and glucagon (18) were determined by radioimmunoassays, and catecholamines were determined by a radioenzymatic method (19). Plasma enrichment of [^{2}H]glucose, [^{13}C]glucose, and [^{13}C]glycerol was determined by gas chromatography–mass spectrometry (20) after derivatization with butane boronic acid in pyridine and acetic anhydride (21). Plasma concentrations of lactate, glycerol, and alanine were determined in samples deproteinized with 6% (wt/vol) perchloric acid (1 ml plasma + 1 ml of perchloric acid) with an enzymatic assay adapted for the Technicon AutoAnalyzer (Technicon, Nashville, TN) (22).

Calculations. Renal plasma and blood flows; renal glucose fractional extraction, uptake, and production; and systemic glucose appearance rates were calculated using formulas described in a separate publication (7). Use of monocompartmental equations can be associated with as much as 20% underestimation of the rate of appearance of glucose under conditions in which there are large and rapid changes in plasma specific activity, such as during hyperinsulinemia (23,24). 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. The net renal balance of each substrate (plasma lactate, glycerol, and alanine) was calculated with the following formula: Net substrate balance = $(C_a - C_{rv}) \times$ RPF, where C_a is arterial plasma substrate concentration, C_{rv} is left or right renal vein plasma substrate concentration, and RPF is unilateral renal plasma flow, as previously described (7). The contribution of the kidney to the rate of appearance of [^{13}C]glucose derived from glycerol ($GNG_{(k)}$) was calculated as in the following, where plasma enrichments of [^{13}C]glucose and [^{13}C]glycerol are represented as [^{13}C-Glu]PE and [^{13}C-Gly]PE, respectively, and glucose concentration as [Glu]:

$$GNG_{(k)} = \frac{RPF \times \{([^{13}C\text{-Glu}]\ PE_{rv} \times [Glu]_{rv}) - (1 - FE_g) \times [^{13}C\text{-Glu}]\ PE_a \times [Glu]_a\}}{2 \times ([^{13}C\text{-Gly}]\ PE_a)}$$

The numerator in the formula represents left or right renal vein (rv) plasma [^{13}C]glucose enrichments (%) in excess of that anticipated from the known arterial (a) plasma [^{13}C]glucose enrichment (%) and the fractional extraction (FE_g) of [^{3}H]glucose (i.e., that [^{13}C]glucose that has been newly generated in the kidney). The denominator in the formula is the precursor pool (arterial glycerol) plasma enrichment (%), taking into account the fact that 2 mol of glycerol are required to generate 1 mol of glucose. This formula underestimates actual renal gluconeogenesis from glycerol to the extent that the kidney metabolizes newly synthesized [^{13}C]glucose. Furthermore, because of isotope dilution within the renal triose phosphate pool (2), measurements of gluconeogenesis using arterial glycerol tracer concentration as the precursor pool represent minimum estimates. Assuming that lactate, alanine, and glycerol extracted by the kidney are completely converted to glucose, a maximal estimate of the overall contribution of these precursor substrates to RGP was calculated by dividing the rate of net renal substrate uptake by two to account for the incorporation of the C-3 precursors into the C-6 glucose molecule. Percent contribution of each substrate was then obtained by dividing individual precursor data by tracer-determined RGP and multiplying by 100.

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

RESULTS

Unilateral renal plasma flow was 4.4 ± 0.4 ml · kg^{-1} · min^{-1} in the baseline and did not change during hypoglycemia (4.8 ± 0.2 ml · kg^{-1} · min^{-1}; NS). Arterial plasma glucose decreased from a mean baseline value of 5.3 ± 0.1 to 2.2 ± 0.1 mmol/l ($P < 0.01$), and plasma insulin increased from 46 ± 5 to 1,050 ± 50 pmol/l ($P < 0.01$) during the last 30 min of the experimental period. Systemic and renal (right and left) glucose kinetics and plasma hormone concentrations after the 120-min hypoglycemic clamp are summarized in Table 1 and have been detailed elsewhere (7). Arterial plasma lactate concentration increased from 858 ± 214 to 3,238 ± 279 µmol/l; left renal vein lactate, from 585 ± 145 to 2,342 ± 250 µmol/l; and right renal vein lactate, from 702 ± 270 to 2,634 ± 236 µmol/l (all $P < 0.01$). Renal fraction extraction of lactate did not change in the left (0.32 ± 0.06 vs. 0.28 ± 0.04; NS) or the right kidney (0.18 ± 0.03 to 0.19 ± 0.05; NS). Left renal net uptake of lactate increased from 1.20 ± 0.40 to 4.30 ± 0.40 µmol · kg^{-1} · min^{-1}, and right renal net uptake of lactate increased from 0.70 ± 0.10 to 2.90 ± 0.60 µmol · kg^{-1} · min^{-1} (all $P < 0.01$) during the experimental period (Fig. 1). Arterial plasma alanine concentration did not change (325 ± 80 vs. 304 ± 70 µmol/l; NS), whereas left renal vein alanine decreased from 315 ± 55 to 244 ± 45 µmol/l, and right renal vein alanine decreased from 334 ± 37 to 179 ± 60 µmol/l (both $P < 0.01$). Renal alanine fractional extraction was 0 at baseline and increased to 0.20 ± 0.04 in the left kidney and 0.41 ± 0.05 in the right kidney ($P < 0.05$ vs. baseline). As a result, neutral renal alanine balance switched to a net uptake of 0.29 ± 0.07 (left) and 0.60 ± 0.03 (right) µmol · kg^{-1} · min^{-1} ($P < 0.05$ vs. baseline) during the experimental period (Fig. 2).

Arterial plasma glycerol concentration increased from 106 ± 5 to 293 ± 52 µmol/l; left renal vein glycerol, from 56 ± 5 to 212 ± 25 µmol/l; and right renal vein glycerol, from 74 ± 7 to 226 ± 36 µmol/l (all $P < 0.01$). Renal fractional extraction of glycerol decreased from 0.47 ± 0.07 to 0.28 ± 0.05 in the left kidney and from 0.30 ± 0.04 to 0.23 ± 0.03 in the right kidney ($P < 0.05$ vs. baseline). However, left renal net uptake of glyc-

TABLE 1
Plasma glucose, endogenous glucose appearance (R_a), right and left RGP, and plasma insulin, glucagon, and catecholamine concentrations in the postabsorptive period and after 120-min hypoglycemic clamp in conscious dogs

	Postabsorptive	Hypoglycemia
Plasma glucose (µmol/ml)		
Arterial	5.30 ± 0.1	2.20 ± 0.1*
Right renal vein	5.30 ± 0.1	2.50 ± 0.1†
Left renal vein	5.30 ± 0.1	5.40 ± 0.1
Glucose R_a (µmol · kg^{-1} · min^{-1})	16.1 ± 0.4	24.2 ± 0.6*
Left RGP (µmol · kg^{-1} · min^{-1})	1.7 ± 0.4	17.0 ± 2.0*
Right RGP (µmol · kg^{-1} · min^{-1})	0.6 ± 0.2	3.2 ± 0.2*
Insulin (pmol/l)	46.0 ± 5.0	1,050.0 ± 50.0*
Glucagon (pg/ml)	52.0 ± 2.0	156.0 ± 12.0*
Epinephrine (pg/ml)	130.0 ± 8.0	1,825.0 ± 50.0*
Norepinephrine (pg/ml)	129.0 ± 6.0	387.0 ± 15.0*

Left renal vein glucose concentration and left renal glucose production rates reflect values attained via direct intrarenal dextrose infusion. *$P < 0.01$ vs. postabsorptive; †$P < 0.01$ vs. postabsorptive and arterial concentration.

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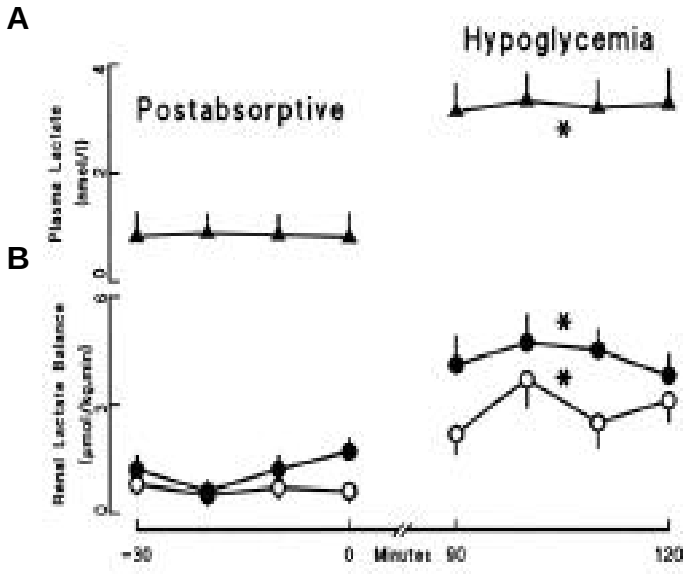

FIG. 1. Plasma lactate concentration (*A*) and net renal lactate balance (*B*) (left, ●; right, ○) in the postabsorptive period (*t* = –30 to 0 min) and during systemic hypoglycemia with simultaneous left renal normoglycemia (*t* = 90–120 min). **P* < 0.01, mean values during last 30 min of experimental period versus mean baseline values.

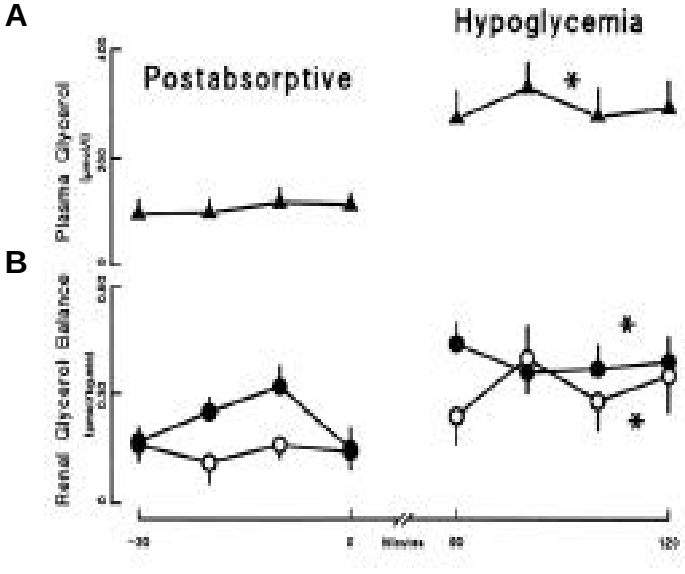

FIG. 3. Plasma glycerol concentration (*A*) and net renal glycerol balance (*B*) (left, ●; right, ○) in the postabsorptive period (*t* = –30 to 0 min) and during systemic hypoglycemia with simultaneous left renal normoglycemia (*t* = 90–120 min); **P* < 0.01, mean values during last 30 min of experimental period versus mean baseline values.

erol increased from 0.22 ± 0.04 to 0.39 ± 0.03 μmol · kg^{-1} · min^{-1} and right renal net uptake of glycerol increased from 0.14 ± 0.02 to 0.32 ± 0.04 μmol · kg^{-1} · min^{-1} (all $P < 0.01$) during the experimental period (Fig. 3). Table 2 summarizes tracer-determined systemic and renal glycerol kinetics. Whole-body glycerol appearance rate and gluconeogenesis from glycerol increased by ~25% during the last 30 min of the experimental period. Renal conversion of [^{13}C]glycerol to glucose increased equally by twofold in the right hypoglycemic or left normoglycemic kidney.

Net renal gluconeogenic precursor uptake increased threefold (from 1.5 ± 0.4 to 5.0 ± 0.4 μmol · kg^{-1} · min^{-1}) in the left kidney and fourfold (from 0.9 ± 0.2 to 3.80 ± 0.4 μmol · kg^{-1} · min^{-1}) in the right kidney ($P < 0.01$). In the baseline period, renal lactate uptake could account for ~40% (~36% in the left and ~58% in the right) of RGP, whereas it was responsible for ~60% (~72% in the left and ~46% in the right) of RGP during hypoglycemia. Renal alanine uptake was negligible in the baseline and could account for ~4% (left) and ~10% (right) of RGP during hypoglycemia. Renal glycerol uptake could account for ~6% (left) and ~12% (right) of RGP in the baseline period and for ~6% of glucose produced by either the left or right hypoglycemic kidney (Fig. 4).

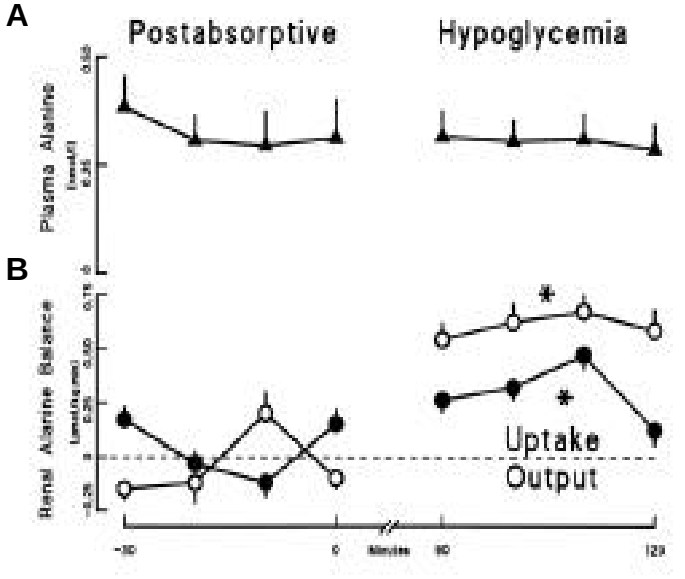

FIG. 2. Plasma alanine concentration (*A*) and net renal alanine balance (*B*) (left, ●; right, ○) in the postabsorptive period (*t* = –30 to 0 min) and during systemic hypoglycemia with simultaneous left renal normoglycemia (*t* = 90–120 min). Values above and below the dotted line indicate net uptake and output, respectively. **P* < 0.01, mean values during last 30 min of experimental period versus mean baseline values.

DISCUSSION

The present study confirms previous findings in dogs (2,4) and in humans (1,3) indicating that prolonged insulin-induced hypoglycemia is accompanied by enhanced gluconeogenesis and further demonstrates that utilization of gluconeogenic precursors by the kidney, particularly lactate, is increased, possibly by counterregulatory hormones. Renal conversion of circulating lactate, glycerol, and to a lesser extent, alanine to glucose could account for nearly 71% of glucose carbons released in the renal vein after 120 min of insulin infusion with sustained hypoglycemia. The absolute fourfold increase in the contribution of these gluconeogenic precursors to glucose production by the kidney was not significantly affected by normalization of renal plasma glucose concentration during systemic hypoglycemia. Although these results contrast with the prevailing notion that hepatic gluconeogenesis is the primary process by which glucose production is sustained as hypoglycemia is prolonged (1–4), past studies are limited in that they did not consider the

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TABLE 2
Whole-body glycerol appearance (R_a), gluconeogenesis (GNG_{WB}), and renal gluconeogenesis from glycerol (right and left GNG_R) in the postabsorptive period and after 120-min hypoglycemic clamp in conscious dogs

	Postabsorptive	Hypoglycemia
Whole-body R_a ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	6.0 ± 0.5	7.7 ± 0.7*
GNG_{WB} ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	1.24 ± 0.04	1.61 ± 0.23*
Left GNG_R ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	0.13 ± 0.04	0.30 ± 0.10*
Right GNG_R ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	0.11 ± 0.03	0.25 ± 0.05*

*$P < 0.05$ vs. postabsorptive.

role of the kidney. By measuring substrate concentrations in plasma sampled directly from renal veins with simultaneous multiple tracer infusion technique, our data complement previous findings (2,4) and provide strong evidence that renal utilization of three-carbon precursor substrates becomes an important additional source of glucose in sustained hypoglycemia. A discrepancy of ~1.1 mg · kg^{-1} · min^{-1} between systemic glucose appearance measured by tracer dilution technique (~6.1 mg · kg^{-1} · min^{-1}) and net hepatic glucose balance measured by arteriovenous difference (~5.0 mg · kg^{-1} · min^{-1}), and the fact that the maximum estimated percent contribution of three-carbon precursor uptake and conversion to glucose by the liver could account for only ~60% of tracer-determined glucose produced after 3 h of hypoglycemia (12% of net hepatic output and 22% of tracer-determined glucose appearance were unaccounted for) were readily apparent in data published by Frizzell et al. (2). Moreover, a difference of ~1.2 mg · kg^{-1} · min^{-1} between tracer-determined glucose production and net hepatic glucose output during high-dosage insulin (8 mU · kg^{-1} · min^{-1})–induced hypoglycemia, and the observation that hepatic precursor uptake and conversion to glucose could only account for 3.8 of the 5.2 mg · kg^{-1} · min^{-1} of tracer-determined glucose production further support the possibility of an extrahepatic source of glucose (4). These discrepancies cannot be entirely explained by the fact that any contribution from precursors produced within the liver was omitted by the techniques used to estimate gluconeogenesis (2,4). Similar analyses of human data are lacking because the independent roles of hepatic and renal gluconeogenesis and glycogenolysis to systemic glucose appearance in conditions of hypoglycemia have not yet been examined (3).

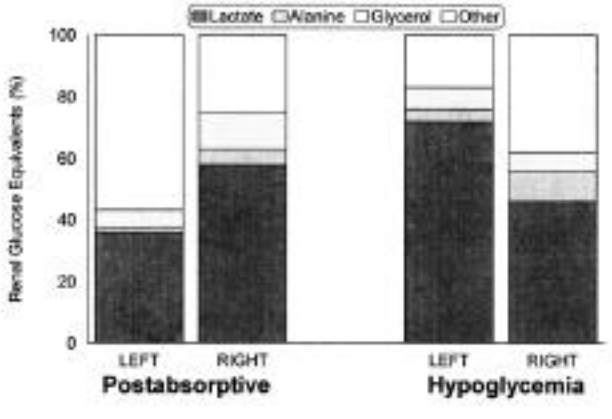

FIG. 4. Estimated percent contribution of circulating lactate, alanine, and glycerol to left and right renal glucose production in the postabsorptive period and during the last 30 min of the experimental period with systemic hypoglycemia and left renal normoglycemia. Data are calculated assuming that net renal uptake of lactate, alanine, and glycerol is entirely diverted toward gluconeogenesis and that tracer-determined renal glucose production represents 100% gluconeogenesis. Percent contribution of "other" reflects renal glucose production unaccounted for by these three-carbon precursor substrates.

The biochemical capacity and physiologic reserve of the kidney to form glucose from noncarbohydrate precursors are well documented (8–12). Our results indicate that renal conversion of circulating lactate, glycerol and alanine to glucose, which may account for as much as ~50% of RGP in the postabsorptive state in conscious dogs, is responsible for ~71% of glucose produced by the kidney during hypoglycemia. Therefore, renal gluconeogenesis from these three-carbon precursors represents ~42% of endogenously produced glucose during prolonged hypoglycemia (i.e., 71% of the kidney's 60% contribution to systemic glucose appearance). According to our data, circulating lactate represents a major contributor to RGP, both in the postabsorptive state and during hypoglycemia. Despite the fact that renal fractional extraction of lactate remained the same, net uptake of lactate by the kidney increased three- to fourfold during hypoglycemia and was not prevented by normalization of renal plasma glucose concentration. These findings agree with previous observations indicating that lactate transport across plasma membranes is not hormonally mediated (25) and support the concept that an increase in renal lactate uptake may have been the result of the elevation in plasma lactate rather than a direct effect of counterregulatory hormones on the kidney. An increase in the overall gluconeogenic efficiency in the kidney, suggested by the rise in the rate of [^{13}C]glucose derived from [^{13}C]glycerol, simultaneous with increments in lactate supply, could sustain augmented rates of gluconeogenesis, even if renal fractional extraction of lactate is not increased. Therefore, our data are consistent with the theory that increments in lactate release from peripheral tissues and in lactate availability to the kidney represent a possible mechanism by which renal gluconeogenesis is enhanced during hypoglycemia (12).

The rates of renal gluconeogenesis from lactate determined in our studies should not be viewed, however, in absolute terms, for various reasons. First, estimation of lactate incorporation into glucose by arteriovenous concentration differences represents a maximum possible contribution and assumes that all circulating lactate extracted by the kidney is diverted into gluconeogenesis. Second, although the amount of intracellularly derived lactate available for gluconeogenesis is likely to be negligible, since lactate formation in the kidney is limited to the distal nephron whereas gluconeogenesis occurs exclusively in proximal tubular cells (12), these techniques do not account for any intracellular sources. Finally, the use of plasma rather than blood lactate concentration together with the fact that circulating pyruvate is not included in the calculation will underestimate the net lactate (pyruvate) balance by an additional ~10–15% (2). Nonetheless, the observation that renal lactate uptake and its

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percent contribution to glucose production account for the largest increase (40–60% of RGP) during hypoglycemia is of particular interest, especially in view of the facts that increased lactate recycling in diabetes is presumed to occur exclusively in the liver (26,27) and lactate conversion to glucose in the liver was recently shown to be inhibited by antihyperglycemic agents used in the treatment of diabetic patients (28). Whether renal gluconeogenesis contributes to lactate recycling in diabetes, however, and whether the process in the kidney, analogous to the liver, is responsive to these drugs is currently unknown.

Conversion of alanine to glucose by the kidney, unlike that converted by the liver, is negligible in the postabsorptive period, and increases only slightly during hypoglycemia to represent ~4% and ~10% of glucose produced in the left and right kidneys, respectively. Because the arterial alanine concentration did not change significantly during hypoglycemia, an increase in alanine fractional extraction by the kidney, similar to that described in the liver (29), was responsible, at least in part, for the mild increase in alanine conversion to glucose. Although we did not determine the contribution of other amino acids, particularly glutamine, to renal gluconeogenesis, these results raise the possibility that uptake and conversion of amino acids to glucose by the kidney, analogous to the liver (16), may be hormonally controlled. The observation that alanine contributes minimally to RGP agrees with results from earlier in vitro studies indicating negligible activity of the enzyme alanine aminotransferase in the kidney, as opposed to its abundance in hepatocytes (30), and is consistent with data obtained in healthy subjects suggesting preferential hepatic utilization of alanine and renal utilization of glutamine for gluconeogenesis (31). The present studies further confirm previous findings indicating that glycerol-derived gluconeogenesis accounts for ~10% of systemic and RGP, and that ~20% of total glycerol conversion of glycerol to glucose occurs in the kidney (~10% in each kidney) in postabsorptive dogs (5). Interestingly, however, although the whole-body glycerol appearance rate and conversion to glucose increased only by ~30%, renal uptake and conversion of glycerol to glucose doubled during hypoglycemia. This was true whether glycerol-derived gluconeogenesis was measured by renal tracer extraction and conversion to glucose (minimal estimate) or by arteriovenous concentration difference (maximal contribution). The fact that both the arterial glycerol concentration and the rate of glycerol appearance increased suggests that adipose tissue lipolysis was stimulated by counterregulatory hormones. Our findings demonstrate that, in addition to its contribution to the liver (32), enhanced lipolysis during hypoglycemia is an important source of glycerol to the kidney. Similar to lactate, renal fractional extraction of glycerol did not change significantly during hypoglycemia, though the efficiency of glycerol conversion to glucose and the amount of glycerol supplied to the kidney increased substantially. As a result, renal glycerol uptake increased twofold, though the maximum percent contribution to glucose carbons attributed to glycerol remained the same at 6–12%. These data agree with analogous observations in the liver that suggest that the transport rates of glycerol across the plasma membrane are not hormonally regulated (4), and lend further support to the concept that increments in peripheral substrate release and consequent supply to the kidney, in addition to intracellular events, drive the rates of renal gluconeogenesis during hypoglycemia. Despite the fact that the left kidney was maintained normoglycemic during the entire hypoglycemic experimental period, RGP and precursor substrate extraction were comparable in both kidneys. These observations strongly suggest that counterregulatory hormones, such as catecholamines and glucagon, exert a stimulating effect on renal gluconeogenesis that overrides insulin's inhibitory effect (5). In agreement with recently published data in humans (6,32), our findings suggest that elevations in catecholamines increase renal gluconeogenesis, at least in part, by stimulating adipose tissue lipolysis and peripheral lactate release. Furthermore, since renal lactate uptake has previously been shown to be increased during physiological hyperglucagonemia in dogs (25), and since alanine transport and utilization in hepatocytes are known to be mediated by glucagon (29), the possibility that renal uptake and conversion of lactate and alanine to glucose are enhanced by glucagon during hypoglycemia should not be ruled out.

In summary, we have demonstrated that insulin-induced hypoglycemia in dogs is accompanied by augmented renal gluconeogenesis, and that utilization of gluconeogenic precursors by the kidney, particularly lactate, is stimulated by counterregulatory hormones. Renal conversion of circulating lactate, glycerol, and to a lesser extent, alanine to glucose accounts for ~71% of glucose produced by the kidney, and is responsible for ~42% of systemic glucose production after 120 min of sustained hypoglycemia. Although the mechanisms for augmentation of precursor uptake by the kidney are not clear, the observation that renal fractional extraction of lactate and glycerol did not change suggests that counterregulatory hormones stimulate renal gluconeogenesis, in part, by increasing peripheral substrate release and supply to the kidney. Nevertheless, an increase in the overall gluconeogenic efficiency in the kidney, suggested by the rise in the rate of [^{13}C]glucose derived from [^{13}C]glycerol, supports an intracellular role for counterregulatory hormones. In addition, an elevation in renal fractional extraction of alanine analogous to the liver, albeit small, may have been mediated by glucagon. We conclude that renal conversion of circulating substrate precursors to glucose, particularly lactate, is responsible for a significant fraction of the enhanced gluconeogenesis induced during sustained hypoglycemia.

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ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants DK-49861 and DK-42562.

We are indebted to Dr. Alan Cherrington for helpful discussion of the manuscript and for the glucagon assay. We thank M. Ajmal, R.J. Naukam, and I. Zaitseva for their technical assistance and L. Cersosimo for help in preparation of the manuscript.

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