Effects of Hyperglycemia, Glucagon, and Epinephrine on Renal Glucose Release in the Conscious Dog

Stephanie M. Gustavson, Chang An Chu, Makoto Nishizawa, Doss Neal, Ben Farmer, Ying Yang, E. Patrick Donahue, Paul Flakoll, and Alan D. Cherrington

The role of renal glucose production after an overnight fast and in response to different hormonal conditions has been debated. The aim of this study was to determine whether hyperglycemia, glucagon, or epinephrine can affect renal glucose production. In 18-hour fasted conscious dogs a pancreatic clamp initially fixed insulin and glucagon at basal levels, following which 1 of 4 protocols was instituted. In G+E glucagon (1.5 ng · kg^{-1} · min^{-1}; portally) and epinephrine (50 ng · kg^{-1} · min^{-1}; peripherally) were increased, in G glucagon was increased alone, in E epinephrine was increased alone, and in C neither were increased. In G, E, and C, glucose was infused to match the hyperglycemia in G+E (~250 mg/dL). The average net renal glucose output during the last 2 hours was not different from the basal values in any group. Furthermore, the changes in unidirectional renal glucose production were not significantly different among groups. Therefore, after an overnight fast in the conscious dog, the kidneys do not significantly contribute to overall glucose production or respond to glucagon or epinephrine.

THE KIDNEYS produce glucose solely via gluconeogenesis since they do not store glycogen.[1] After an overnight fast, renal glucose production accounts for approximately 5% to 30% of the total tracer-determined glucose production.[2-9] However, after an overnight fast the kidneys have been shown to consume approximately the same amount of glucose they produce; hence, little or no net glucose output is seen.[3,5-7,9,10] The ability of the kidneys to increase glucose production in response to counterregulatory hormones is currently under debate. McGuinness et al showed that a chronic (70-hour) infusion of glucagon, epinephrine, norepinephrine, and cortisol in conscious dogs caused a 3-fold increase in renal glucose release,[9] even though net renal glucose output did not increase. When all the hormones were chronically elevated again, except for glucagon, renal glucose release did not increase, thereby implying that glucagon was necessary for the increase in renal glucose release.[11] When the hormones were chronically elevated in yet another study, the presence or absence of epinephrine had no effect on renal glucose release, implying that epinephrine was not necessary for an increase in renal glucose production.[12] Cersosimo et al demonstrated that during insulin-induced hypoglycemia in conscious dogs, which stimulated a rise in the counterregulatory hormones, renal glucose release and net renal glucose output both increased significantly[13]and gluconeogenic precursor extraction by the kidney was stimulated.[14] Interestingly, when beta-adrenergic blockade was employed during hypoglycemia, the increase in renal glucose release was prevented, implying that acutely epinephrine may in fact be the cause of the increase in renal glucose output during hypoglycemia.[15] Studies on healthy humans also showed that insulin-induced hypoglycemia resulted in a significant increase in renal glucose release and net renal glucose output,[16,17] and that during hypoglycemia net renal fractional extraction of gluconeogenic substrates increased.[18] Finally, during hypoglycemia in type I diabetic patients, who had blunted glucagon and epinephrine responses, the rise in endogenous glucose production and net renal glucose output that occurred in normal subjects was absent.[19]

When the hormones were studied individually, it was shown that glucagon does not stimulate renal gluconeogenesis in the isolated perfused rat kidney.[20] Moreover, in normal postabsorptive humans (12- to 14-hour fasted), raising plasma glucagon concentrations 3-fold acutely did not effect renal glucose release or uptake.[21]. Although it was also shown that epinephrine does not stimulate renal gluconeogenesis in the isolated perfused rat kidney.[20] catecholamines have been shown to stimulate gluconeogenesis in isolated renal cortical tissue.[22] Interestingly, administration of cyclic adensoine monophosphate (cAMP), but not epinephrine, stimulated gluconeogenesis from lactate and glutamine by isolated human renal proximal tubules.[23] A recent study on streptozotocin-induced diabetic rats showed that the increase in blood glucose after epinephrine infusion was significantly blunted in nephrectomized rats compared to control rats with functional kidneys.[24] In humans, an epinephrine infusion caused a sustained increase in renal glucose release, such that after 3 hours 40% of overall systemic glucose appearance, and essentially all of the increase in systemic glucose release, could be accounted for by renal glucose release.[5] Additionally, epinephrine infusion in humans increased renal gluconeogenesis by enhancing substrate availability (lactate and alanine), fractional extraction (glutamine), and gluconeogenic efficiency.[25,26] Due to the discrepancies in the literature, the aim of the current study was to determine in the dog whether the kidneys are acutely responsive to epinephrine and/or glucagon.

MATERIALS AND METHODS

Animals and Surgical Procedures

Studies were performed on 18 overnight-fasted conscious mongrel dogs of either sex (19 to 26.3 kg; mean, 22.9 kg). Animals were fed

From the Department of Molecular Physiology and Biophysics and Diabetes Research and Training Center, Vanderbilt University, Nashville, TN.

Submitted November 21, 2003; accepted January 31, 2004.

Supported by Grants No. P60-DK-20593, P30-DK-26657, T32-DK-07563, and R37-DK^{-1}8243 from the National Institutes of Health.

Address reprint requests to Stephanie M. Gustavson, PhD, Vanderbilt University Medical Center, Division of Diabetes, Endocrinology, and Metabolism, 715 Preston Research Building, Nashville, TN 37232-6303.

0026-0495/04/5307-0007$30.00/0

doi:10.1016/j.metabol.2004.01.018

once daily a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet No. 5006; Purina Mills, St Louis, MO) comprised of 46% carbohydrate, 34% protein, 14% fat, and 6% fiber based on dry weight. The animals were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days prior to the study, a laparotomy was performed under general anesthesia (15 mg/kg body weight sodium thiopental presurgery; 1.0% isoflurane as an inhalation anesthetic during surgery). In all dogs, an ultrasonic flow probe (Transonic Systems, Ithaca, NY) was positioned around a renal artery. Silastic catheters (Dow Corning, Midline, MI) were inserted into a femoral artery and a renal vein for blood sampling and into the splenic and jejunal veins for intraportal hormone delivery. The catheters were filled with heparinized saline (200 U/mL; Abbott Laboratories, North Chicago, IL) and their free ends were knotted. The free ends of the catheters and the flow probe lead were placed in subcutaneous pockets until the study day. Animals were studied only if the following criteria were met prior to the study: (1) leukocyte count less than 18,000/mm^3, (2) hematocrit greater than 35%, (3) good appetite, and (4) normal stools. Note that in all dogs ultrasonic flow probes were positioned around the hepatic artery and the portal vein, and Silastic catheters were inserted into a hepatic vein and the portal vein. The hepatic glucose balance data form the basis of a separate report.[27]

On the morning of a study, the Transonic leads and the catheters were exteriorized under local anesthesia (2% lidocaine; Abbott Laboratories). The dog was placed in a Pavlov harness, and the contents of the catheters were aspirated, after which the catheters were flushed with saline and subsequently used for blood sampling or infusion. Angiocaths (20 gauge; Becton Dickinson, Sandy, UT) were inserted into the right and left cephalic veins for infusion of [3-^{3}H]glucose (New England Nuclear, Boston, MA) and glucose (20% Dextrose; Baxter Healthcare, Deerfield, IL; or 50% Dextrose; Abbott Laboratories), respectively. An angiocath was also placed in the left saphenous vein for somatostatin (Bachem, Torrance, CA) infusion. If required according to the protocol, an angiocath was placed in the right saphenous vein for peripheral epinephrine (Sigma Chemical, St Louis, MO) infusion.

Experimental Design

Each experiment consisted of a 100-minute tracer equilibration and hormone adjustment period (−140 to −40 minutes) followed by a 40-minute control period (−40 to 0 minutes). During these periods, [3-^{3}H]glucose (~50 μCi′ ~0.50 μCi/min) was infused. In addition, a pancreatic clamp was performed. This involved infusion of somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) through a peripheral vein to inhibit endogenous insulin and glucagon secretion, and replacement of insulin (~250 $\mu U \cdot kg^{-1} \cdot min^{-1}$; Eli Lilly, Indianapolis, IN) and glucagon (0.5 $ng \cdot kg^{-1} \cdot min^{-1}$; Bedford Laboratories, Bedford, OH) intraportally. The insulin infusion rate was varied if necessary during the equilibration period to maintain euglycemia. The control period was followed by a 4-hour experimental period (0 to 240 minutes) during which basal insulin was maintained. Each dog underwent one of 4 experimental protocols. In G+E (n = 4) glucagon (1.5 $ng \cdot kg^{-1} \cdot min^{-1}$; portally) and epinephrine (50 $ng \cdot kg^{-1} \cdot min^{-1}$; peripherally) were elevated, in G (n = 5) glucagon (1.5 $ng \cdot kg^{-1} \cdot min^{-1}$; portally) alone was increased, in E (n = 4) epinephrine (50 $ng \cdot kg^{-1} \cdot min^{-1}$; peripherally) alone was raised, and in C (n = 5) basal glucagon and epinephrine (no epinephrine infusion) were maintained. In the G, E, and C protocols, glucose was infused peripherally to match the plasma glucose seen in G+E (~250 mg/dL). The [3-^{3}H]glucose infusion rate was also varied throughout the experimental period in order to clamp the glucose specific activity, and thereby minimize errors in glucose turnover calculation. Basically, for every increase of 2 $mg \cdot kg^{-1} \cdot min^{-1}$ in total glucose Ra, the [3-^{3}H]glucose infusion rate was increased by an amount equal to the basal infusion rate. This rate of increase was similar to that used in previous studies, and was refined based on the results of the first few dogs of the present study. In addition, in order to prevent a slow decline in glucagon levels, the glucagon infusion rate was increased slightly each hour. In dogs receiving basal glucagon, glucagon infusion was increased from 0.50 to 0.54, 0.58, and 0.62 $ng \cdot kg^{-1} \cdot min^{-1}$ at times 60, 120, and 180 minutes, respectively. In dogs receiving 3-fold basal glucagon, glucagon infusion was increased from 1.5 to 1.62, 1.74, and 1.86 at times 60, 120, and 180 minutes, respectively. In all dogs, mean arterial blood pressure and heart rate were determined throughout the experiment using either a chart recorder with blood pressure transducer (Gould RS3200; Gould, Valley View, OH) or a Digi-Med Blood Pressure Analyzer (Micro-Med, Louisville, KY) and have been previously reported.[27]

Analytical Procedures

The immediate processing of the samples and the measurement of whole blood glucose, glutamine, glutamate, gluconeogenic amino acids (serine, threonine, glycine), and metabolites (lactate, alanine, glycerol) were described previously.[28,29] In addition, plasma levels of glucose, [3-^{3}H]glucose, catecholamines, insulin, glucagon, and cortisol were measured as previously described.[28,29] C-peptide (in plasma to which 500 KIU/mL Trasylol had been added; FBA Pharmaceuticals, New York NY) was determined via disequilibrium double antibody radioimmunoassay (Linco Research, St Charles, MO) with an interassay coefficient of variation of 5%.

Calculations

A transonic flow probe was used to estimate renal blood flow in these studies. Measured renal artery flow was multiplied by 2 in order to obtain total kidney flow. The net renal balances of blood glucose, lactate, alanine, glycerol, glutamine, glutamate, serine, threonine, and glycine were calculated as follows: Net Renal Substrate Balance = (R − A) × RF, where R and A are renal vein and arterial blood substrate concentrations respectively and RF is total renal blood flow. Positive numbers indicate net renal output while negative numbers indicate net renal uptake.

Renal glucose release (RGR) was determined by measuring glucose and [3-^{3}H]glucose in the renal vein and artery. The equation is as follows: RGR = NRGB + RGU; RGU = [(A [3-^{3}H]glucose − RV [3-^{3}H]glucose)/SA] · RPF, where NRGB is net renal glucose balance, RGU is renal glucose uptake, A [3-^{3}H]glucose and RV [3-^{3}H]glucose are the plasma [3-^{3}H]glucose levels in the artery and renal vein, respectively, RPF is total renal plasma flow, SA is specific activity, which is calculated by dividing the arterial plasma [3-^{3}H]glucose level by the arterial plasma glucose level, and RPF is renal plasma flow.

Statistical Analysis

Data are expressed as means ± SE. Basal renal balance data were similar among groups and were therefore pooled into a single value for Table 2. Statistical comparisons were made by 1-way analysis of variance (ANOVA; all figures) and 2-way ANOVA with repeated measures design (all tables except Table 2, which involved no statistical comparisons) run on SigmaStat (SPSS Science, Chicago, IL). Post hoc analysis for 1- and 2-way repeated-measures ANOVA was performed with the Tukey test. Statistical significance was accepted at $P < .05$.

Table 1. Arterial Plasma Levels of Glucose, Insulin, Glucagon, and Epinephrine During the Basal Period and Last 2 Hours of the treatment period in Studies Conducted on 18-Hour Fasted Conscious Dogs Maintained on a Pancreatic Clamp and Exposed to a Rise in Either Glucagon (n = 5), Epinephrine (n = 4), or Both (G + E; n = 4), or Matched Hyperglycemia (n = 5)

Parameter and Group	Basal Period (−40 to 0 min)	Last 2 Hours of Treatment (120 to 240 min)
Arterial plasma glucose (mg/dL)		
C	116 ± 6	249 ± 2*
G	110 ± 3	246 ± 12*
E	113 ± 6	252 ± 9*
G + E	109 ± 5	259 ± 21*
Arterial plasma insulin (μU/mL)		
C	4 ± 1	5 ± 1
G	5 ± 1	6 ± 2*
E	5 ± 1	7 ± 1*
G + E	4 ± 1	6 ± 1*
Arterial plasma glucagon (pg/mL)		
C	44 ± 6	39 ± 5[bd]
G	50 ± 9	74 ± 13*[ac]
E	41 ± 5	35 ± 1[bd]
G + E	41 ± 4	72 ± 10*[ac]
Arterial plasma epinephrine (pg/mL)		
C	195 ± 52	212 ± 64[cd]
G	172 ± 33	184 ± 40[cd]
E	162 ± 55	936 ± 139*[ab]
G + E	186 ± 62	1,059 ± 86*[ab]

NOTE. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated-measures design with a Tukey post-hoc analysis.

*$P < .05$ for basal *v* last 2 hours within a group.

[a]$P < .05$ *v* C; [b]$P < .05$ *v* G; [c]$P < .05$ *v* E; [d]$P < .05$ *v* G + E.

RESULTS

Glucose and Hormone Levels

In all 4 groups, plasma glucose levels rose from approximately 110 mg/dL to about 250 mg/dL (Table 1). The plasma insulin levels changed minimally from basal and were not significantly different among groups (Table 1). Arterial plasma C-peptide levels, measured as an index of endogenous insulin secretion, were low and did not change in any group (data not shown), thereby confirming continued inhibition of insulin release even in the presence of hyperglycemia. Arterial plasma glucagon levels rose similarly in the protocols in which the glucagon infusion was increased (G and G+E), but remained basal in the other protocols (Table 1). Arterial plasma epinephrine levels rose similarly in the protocols in which epinephrine was infused (E and G+E), but remained basal when the catecholamine was not infused (Table 1). Arterial cortisol levels as well as arterial norepinephrine levels remained basal in all groups throughout the studies (data not shown).

Glucose Metabolism

Table 2 portrays the pooled net renal balance data from the 4 groups in the basal period during a pancreatic clamp after an

Table 2. Average, Pooled, Value During the Basal Period of All Studies Conducted on 18-Hour Fasted Conscious Dogs Maintained on a Pancreatic Clamp (N = 18)

Parameter	Average, Pooled, Basal Period (−40 to 0 min)	
	Uptake	Output
Net renal glucose balance ($mg \cdot kg^{-1} \cdot min^{-1}$)	−0.19 ± 0.04	
Net renal lactate balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	−1.72 ± 0.13	
Net renal glycerol balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	−0.17 ± 0.04	
Net renal glutamine balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	−0.85 ± 0.10	
Net renal glycine balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	−0.40 ± 0.05	
Net renal threonine balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	−0.03 ± 0.04	
Net renal glutamate balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)		0.10 ± 0.03
Net renal alanine balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)		0.32 ± 0.05
Net renal serine balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)		0.63 ± 0.06

NOTE. Data are expressed as means ± SE. Negative balance refers to net uptake, while positive balance refers to net output.

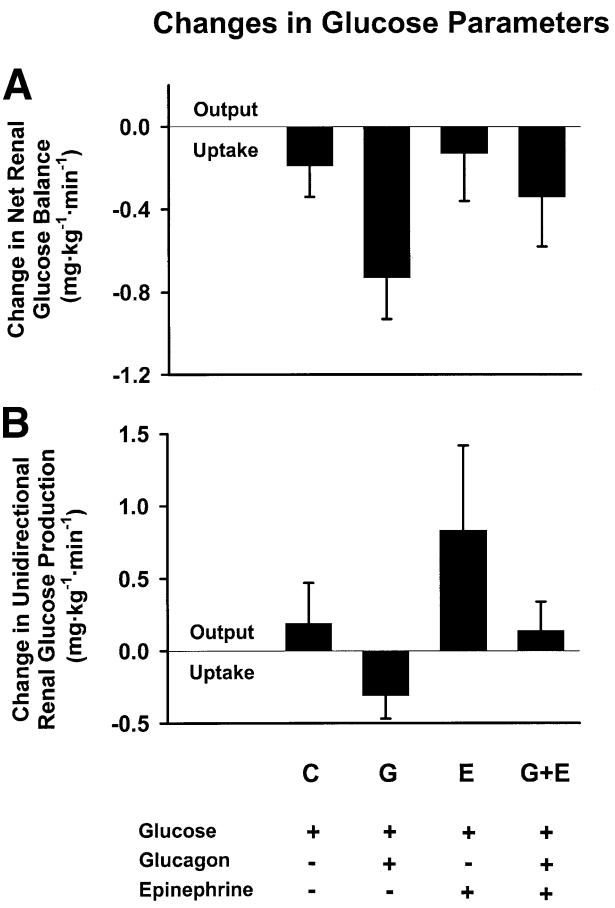

Fig 1. (A) Average net renal glucose balance and (B) average unidirectional renal glucose production in the basal period (−40 to 0 min) subtracted from the average of the last 2 hours of the treatment period (120 to 240 min) in C, G, E, and G+E 18-hour fasted conscious dogs. Data are expressed as mean ± SE. n = 5 for C and G, n = 4 for E and G+E. There was no significant difference among groups.

overnight fast. Net renal glucose uptake tended to increase over time in all groups, with no significant difference among groups, probably due to the increase in the plasma glucose level (Fig 1). Clearly, the elevation in hormone levels did not stimulate net renal glucose output. The pooled basal period value for tracer-determined, unidirectional renal glucose production was minimal (0.00 ± 0.06 mg · kg^{-1} · min^{-1}). Changes in unidirectional renal glucose production also were not significant, although there was a tendency for epinephrine to increase it (Fig 1).

Arterial Lactate Levels and Net Renal Lactate Balance

In the control and glucagon groups, arterial blood lactate levels rose modestly (Table 3). Because net hepatic lactate output increased in both of these groups,[27] the liver is responsible for the increase in arterial lactate levels. In the epinephrine group, arterial lactate levels rose to a markedly greater extent than in C or G. This appears to be the result of increased output from nonhepatic tissues (most likely muscle), as the liver ceased to produce lactate in a net sense[27] in this group. Finally, when both hormones increased concurrently, arterial blood lactate levels rose midway between those obtained with glucagon and epinephrine alone. Net renal lactate uptake increased in all groups, with no difference among the groups (Fig 2).

Arterial Glycerol Levels and Net Renal Glycerol Balance

In both the hyperglycemic control protocol and the glucagon protocol, arterial glycerol levels drifted down (significantly in G, nonsignificantly in C; Table 3). No drift down was seen when epinephrine was given alone or in combination with glucagon. Net renal glycerol uptake did not change significantly in any treatment group (Fig 2).

Arterial Levels of Gluconeogenic Amino Acids and Net Renal Balance of Gluconeogenic Amino Acids

In the hyperglycemic control and epinephrine alone groups, arterial alanine levels rose (Table 3). In both groups involving glucagon infusion, the arterial alanine levels did not change. In contrast to alanine, the arterial levels of the other gluconeogenic amino acids generally fell in each protocol, with no significant differences among groups (Table 3). Nevertheless, the declines were greatest in the presence of glucagon. The changes in net renal balances of the gluconeogenic amino acids were similar among the groups (Figs 3 through 5). Note that after an overnight fast in a net sense 2 of the gluconeogenic amino acids, glutamine and glycine, were taken up by the kidneys, 2 of the amino acids, threonine and glutamate, were neither taken up nor released in significant amounts, and 2 of the amino acids, alanine and serine, were released (Table 2).

DISCUSSION

Our data indicate that after an overnight fast in conscious dogs, renal glucose production is minimal. In fact, in a net sense the kidneys consumed a small amount of glucose in all groups. Such data have been reported previously for the dog[3,5,10] and humans.[5-7] Net renal glucose balance (mg · kg^{-1} · min^{-1}) did not change appreciably in response to hyperglycemia (Δ -0.19 ± 0.15), glucagon (Δ -0.73 ± 0.20), epinephrine (Δ -0.13 ± 0.23), or the combination of the 2 hormones (Δ -0.34 ± 0.24), thus implying that after a brief fast in the dog the kidneys are relatively insensitive to these hormonal or metabolic changes with respect to glucose metabolism.

A recent study suggested that the A-V difference technique may not be sensitive enough to detect changes in renal glucose production should they occur.[30] It has been shown using net renal balance data that there is a negligible contribution of the kidneys to gluconeogenesis in the postabsorptive state.[6] Yet, isotopic studies looking at unidirectional glucose production have found renal glucose production can actually account for approximately 5% to 30% of systemic glucose release during the postabsorptive state.[2,3,5,6,13,31] In our studies we also measured unidirectional renal glucose release, but we did not observe a significant amount of glucose production by the kidneys after an overnight fast during a pancreatic clamp. Hyperglycemia had no effect on unidirectional renal glucose release, which is consistent with a previous finding that the kidneys play a negligible role in plasma glucose disposal during hyperglyce-

Table 3. Arterial Blood Levels of Lactate, Glycerol, and the Gluconeogenic Amino Acids During the Basal Period and Last 2 Hours of the Treatment Period in Studies Conducted on 18-Hour Fasted Conscious Dogs Maintained on a Pancreatic Clamp and Exposed to a Rise in Either Glucagon (n = 5), Epinephrine (n = 4), or Both G + E; n = 4), or Matched Hyperglycemia (n = 5)

Parameter and Group	Basal Period (−40 to 0 min)	Last 2 Hours of Treatment (120 to 240 min)
Arterial blood lactate (μmol/L)		
C	772 ± 113	1445 ± 206*cd
G	790 ± 74	1394 ± 95*cd
E	998 ± 36	3778 ± 174*abd
G + E	699 ± 231	2570 ± 355*abc
Arterial blood glycerol (μmol/L)		
C	53 ± 2b	34 ± 6d
G	93 ± 14a	65 ± 14*
E	70 ± 10	72 ± 6
G + E	70 ± 10	77 ± 19a
Arterial blood alanine (μmol/l)		
C	380 ± 31	574 ± 59*bd
G	377 ± 72	383 ± 59ac
E	419 ± 66	588 ± 66*bd
G + E	347 ± 32	334 ± 36ac
Arterial blood serine (μmol/L)		
C	141 ± 4	110 ± 6*
G	173 ± 19d	108 ± 6*
E	147 ± 27	109 ± 18*
G + E	121 ± 7b	82 ± 1*
Arterial blood threonine (μmol/L)		
C	255 ± 18	216 ± 25*
G	271 ± 25	193 ± 31*
E	246 ± 46	199 ± 46*
G + E	226 ± 30	150 ± 15*
Arterial blood glutamate (μmol/L)		
C	57 ± 8	46 ± 12
G	61 ± 11	62 ± 13
E	53 ± 16	37 ± 14*
G + E	52 ± 4	34 ± 5*
Arterial blood glutamine (μmol/L)		
C	892 ± 38	838 ± 48
G	952 ± 67	745 ± 52*
E	746 ± 159	668 ± 138
G + E	864 ± 64	642 ± 40*
Arterial blood glycine (μmol/L)		
C	240 ± 24	196 ± 17*
G	261 ± 17	157 ± 8*
E	248 ± 41	165 ± 20*
G + E	256 ± 28	146 ± 14*

NOTE. Data are expressed as mean ± SE. Statistical comparisons were made by 2-way ANOVA with repeated-measures design with a Tukey post-hoc analysis.

*$P < .05$ for basal *v* last 2 hours within a group.

[a]$P < .05$ *v* C; [b]$P < .05$ *v* G; [c]$P < .05$ *v* E; [d]$P < .05$ *v* G + E.

mia in conscious dogs.[32] Glucagon had no effect on renal glucose release in the present study, which is also consistent with previous experiments.[20,21] It appears that epinephrine alone may have increased unidirectional renal glucose release modestly (although this was not statistically significant), which other investigators have also observed.[5,22] It is possible that the previous investigators observed a larger effect of the catecholamine on renal glucose production than we did because arterial epinephrine levels were elevated to 15-fold basal in their study,[5] whereas in the present study they were only elevated 6-fold. However, epinephrine treatment combined with glucagon infusion did not result in an increase in renal glucose release, suggesting either that epinephrine's effect in this study was minimal, or that glucagon might somehow reduce the effects of epinephrine.

Further evidence that glucagon, epinephrine, and hyperglycemia did not significantly affect renal glucose metabolism in these studies can be found in the net renal balance of the gluconeogenic precursors. If, for example, epinephrine had significantly stimulated renal glucose production, but the effect

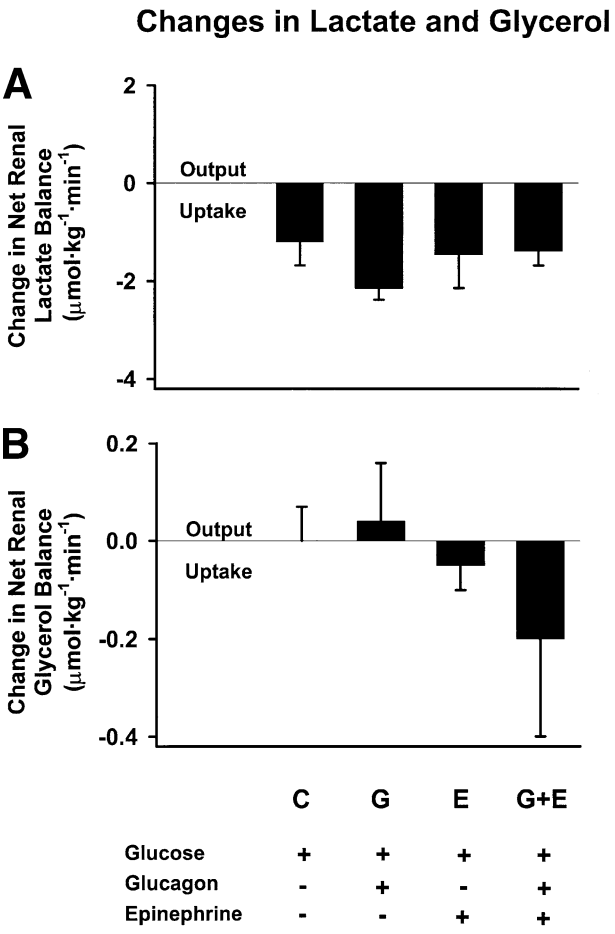

Fig 2. (A) Average net renal lactate balance and (B) average net renal glycerol balance in the basal period (−40 to 0 min) subtracted from the average of the last 2 hours of the treatment period (120 to 240 min) in C, G, E, and G+E 18-hour fasted conscious dogs. Data are expressed as mean ± SE. n = 5 for C and G, n = 4 for E and G+E. There was no significant difference among groups.

could not be determined conclusively due to insensitivity of the methods, one would have expected an increase in net renal uptake of the gluconeogenic precursors in the epinephrine group. Such was not observed. Renal uptake of lactate, glycerol, glutamine, and glycine, the primary gluconeogenic precursors used by the kidneys,[33-35] did not increase more in response to epinephrine administration than to any of the other conditions studied.

Interestingly, not all of the gluconeogenic amino acids were taken up in a net sense by the kidneys after an overnight fast. Notably, both alanine and serine were released from the kidneys in a net sense, threonine and glutamate were neither released nor taken up, and glutamine and glycine were taken up in a net sense. These observations agree with previous findings in 12- to 14-hour fasted humans.[36,37]

Even though in the present studies the kidneys did not produce an appreciable amount of glucose and did not respond to different hormones, other studies have found that the kidneys can respond to different metabolic situations. For example, after removal of the liver during hepatic transplantation, endogenous glucose release does not drop to zero, and 1 hour after the removal endogenous glucose release was only reduced by approximately 50%.[31] In addition, patients with renal failure have a propensity to develop hypoglycemia.[38] Furthermore, as described in detail in the introduction, hypoglycemia has been shown to influence renal glucose release. Other situations in which the kidneys appear important to glucose homeostasis are during diabetes and fasting, 2 insulin-deficient states. Increased renal gluconeogenic enzyme activity and increased renal glucose release have been consistently demonstrated in diabetic animals (for review see Gerich et al[7]). Additionally, Meyer et al showed that in type II diabetic patients the increments in postabsorptive hepatic and renal glucose release were similar ($\sim$0.45 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}), although renal uptake also increased, resulting in continued net renal glucose uptake.[39] As a side note, glucose output in response to epinephrine infusion

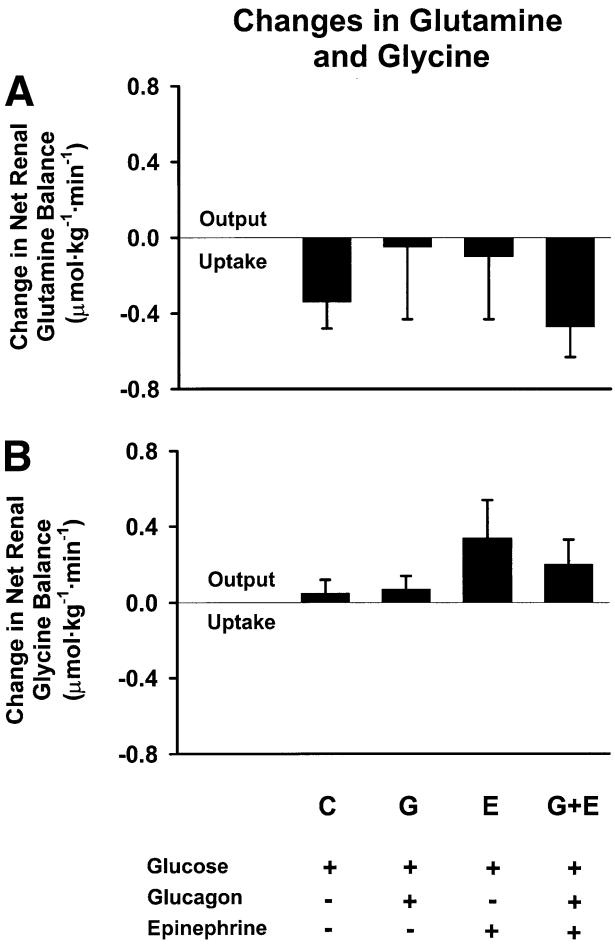

Fig 3. (A) Average net renal glutamine balance and (B) average net renal glycine balance in the basal period (-40 to 0 min) subtracted from the average of the last 2 hours of the treatment period (120 to 240 min) in C, G, E, and G+E 18-hour fasted conscious dogs. Data are expressed as mean ± SE. n = 5 for C and G, n = 4 for E and G+E. There was no significant difference among groups.

was significantly higher in perfused kidney of diabetic rats compared with normal rats, suggesting that the adrenergic stimulation of renal glucose output may be enhanced in diabetes.[24] As fasting progresses, the kidneys increase their net contribution to overall glucose release.[2,40,41] More specifically, it was shown that renal glucose release increased 2.5-fold after a 60-hour fast compared with overnight fasted humans, while renal glucose uptake decreased.[2] Ironically, the kidney has also been shown to increase renal glucose release postprandially, while hepatic glucose release is suppressed.[42] A proposed explanation for the increase in renal glucose production postprandially is that this allows efficient liver glycogen replenishing by permitting substantial suppression of hepatic glucose release.[42] Finally, 2 independent groups have demonstrated that the kidneys respond to insulin, in that insulin suppresses renal glucose release and increases renal glucose uptake in humans[4,43] and dogs.[3] In fact, one study indicated that renal gluconeogenesis is more sensitive to insulin than is hepatic gluconeogenesis, as

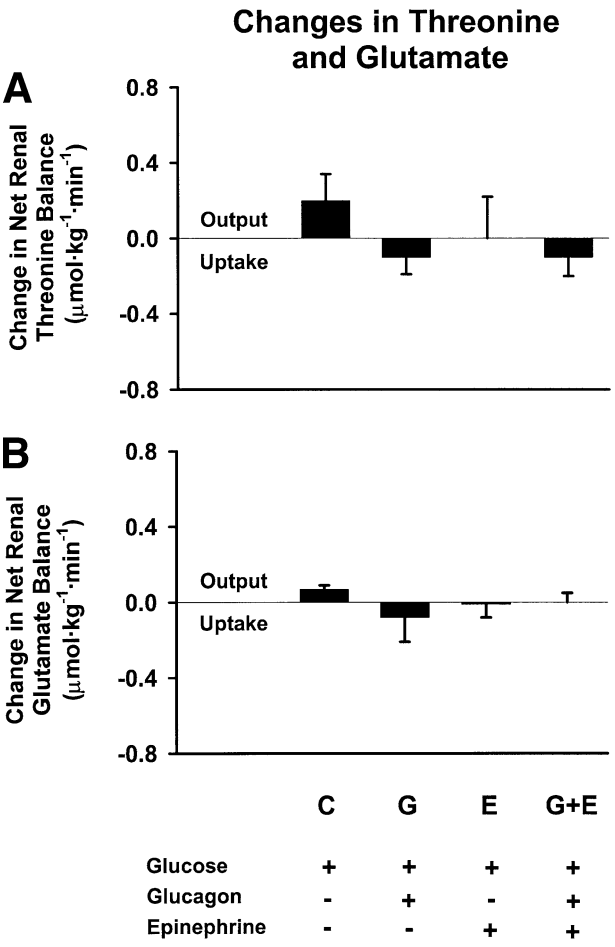

Fig 4. (A) Average net renal threonine balance and (B) average net renal glutamate balance in the basal period (−40 to 0 min) subtracted from the average of the last 2 hours of the treatment period (120 to 240 min) in C, G, E, and G+E 18-hour fasted conscious dogs. Data are expressed as mean ± SE. n = 5 for C and G, n = 4 for E and G+E. There was no significant difference among groups.

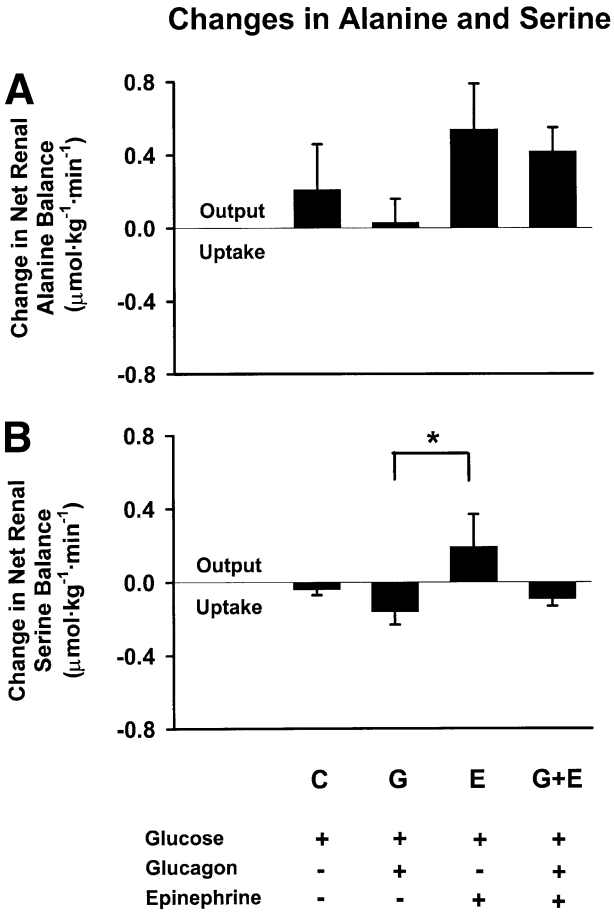

Fig 5. (A) Average net renal alanine balance and (B) Average net renal serine balance in the basal period (−40 to 0 min) subtracted from the average of the last 2 hours of the treatment period (120 to 240 min) in C, G, E, and G+E 18-hour fasted conscious dogs. Data are expressed as mean ± SE. n = 5 for C and G, n = 4 for E and G+E. There was no significant difference among groups for (A). *Significant difference ($P < .05$) between G and E in (B).

gluconeogenesis and net lactate uptake in the kidneys were decreased much faster than in the liver.[44] However, a recent study revealed that acute exogenous insulin replacement in type II diabetic patients was only able to suppress hepatic, not renal, glucose release.[45]

In conclusion, net renal glucose production was minimal in conscious dogs maintained on a pancreatic clamp after an overnight fast. Physiological elevations in glucagon, epinephrine, or a control hyperglycemia did not significantly alter renal glucose metabolism, although elevations in these parameters significantly affected hepatic glucose metabolism as we showed in our previous study.[27] It is clear that in the conscious dog while the kidneys may respond to several metabolic situations, they are not nearly as sensitive as the liver to elevations in glucagon, epinephrine, or glucose after an overnight fast. A final point is that under stress conditions such changes in glucagon and epinephrine would undoubtedly be accompanied by changes in insulin, resulting in a smaller increment in

plasma glucose, and it remains to be seen whether in the presence of hyperinsulinemia and lower glucose levels the interaction of glucagon and epinephrine on renal glucose production would be altered.

ACKNOWLEDGMENT

We wish to thank Margaret Converse, Wanda Snead, Eric Allen, Angela Penaloza, and Jon Hastings for their excellent technical support.

REFERENCES

1. Biava C, Grossman A, West M: Ultrastructural observations on renal glycogen in normal and pathologic human kidneys. Lab Invest 15:330-356, 1966
2. Ekberg K, Landau BR, Wajngot A, et al: Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48:292-298, 1999
3. Cersosimo E, Judd RL, Miles JM: Insulin regulation of renal glucose metabolism in conscious dogs. J Clin Invest 93:584-2589, 1994
4. Cersosimo E, Garlick P, Ferretti J: Insulin regulation of renal glucose metabolism in humans. Am J Physiol 276:E78-84, 1999
5. Stumvoll M, Chintalapudi U, Perriello G, et al: Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. J Clin Invest 96:2528-2533, 1995
6. Stumvoll M, Meyer C, Mitrakou A, et al: Renal glucose production and utilization: New aspects in humans. Diabetologia 40:749-757, 1997
7. Gerich JE, Meyer C, Woerle HJ, et al: Renal gluconeogenesis: Its importance in human glucose homeostasis. Diabetes Care 24:982-391, 2001
8. Ekberg K, Landau BR, Wajngot A, et al: Splanchnic and renal glucose production in the postabsorptive state in healthy humans. Diabetologia 41:A202, 1998 (abstr)
9. McGuinness OP, Fugiwara T, Murrell S, et al: Impact of chronic stress hormone infusion on hepatic carbohydrate metabolism in the conscious dog. Am J Physiol 265:E314-322, 1993
10. Cherrington AD: Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 48:1198-1214, 1999
11. McGuinness OP, Burgin K, Moran C, et al: Role of glucagon in the metabolic response to stress hormone infusion in the conscious dog. Am J Physiol 266:E438-447, 1994
12. McGuinness OP, Shau V, Benson EM, et al: Role of epinephrine and norepinephrine in the metabolic response to stress hormone infusion in the conscious dog. Am J Physiol 273:E674-681, 1997
13. Cersosimo E, Molina PE, Abumrad NN: Renal glucose production during insulin-induced hypoglycemia. Diabetes 46:643-646, 1997
14. Cersosimo E, Molina PE, Abumrad NN: Renal lactate metabolism and gluconeogenesis during insulin-induced hypoglycemia. Diabetes 47:1101-1106, 1998
15. Cersosimo E, Zaitseva IN, Ajmal M: Effects of beta-adrenergic blockade on hepatic and renal glucose production during hypoglycemia in conscious dogs. Am J Physiol 275:E792-797, 1998
16. Meyer C, Dostou JM, Gerich JE: Role of the human kidney in glucose counterregulation. Diabetes 48:943-948, 1999
17. Cersosimo E, Garlick P, Ferretti J: Renal glucose production during insulin-induced hypoglycemia in humans. Diabetes 48:261-266, 1999
18. Cersosimo E, Garlick P, Ferretti J: Renal substrate metabolism and gluconeogenesis during hypoglycemia in humans. Diabetes 49: 1186-1193, 2000
19. Cersosimo E, Garlick P, Ferretti J: Abnormal glucose handling by the kidney in response to hypoglycemia in type 1 diabetes. Diabetes 50:2087-2093, 2001
20. Bowman RH: Gluconeogenesis in the isolated perfused rat kidney. J Biol Chem 245:1604-1612, 1970
21. Stumvoll M, Meyer C, Kreider M, et al: Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. Metabolism 47:1227-1232, 1998
22. Kurokawa K, Massry SG: Evidence for stimulation of renal gluconeogenesis by catecholamines. J Clin Invest 52:961-964, 1973
23. Conjard A, Martin M, Guitton J, et al: Gluconeogenesis from glutamine and lactate in the isolated human renal proximal tubule: Longitudinal heterogeneity and lack of response to adrenaline. Biochem J 360:371-377, 2001
24. Ikeda T, Iwata K: Enhanced adrenergic mechanisms in glucose output from the kidney of diabetic rats. Metabolism 52:772-475, 2003
25. Meyer C, Stumvoll M, Welle S, et al: Relative importance of liver, kidney, and substrates in epinephrine-induced increased gluconeogenesis in humans. Am J Physiol Endocrinol Metab 285:819-826, 2003
26. Meyer C, Stumvoll M, Welle S, et al: Sites, substrates, and mechanisms of epinephrine stimulated glucose production in humans. Diabetes 47:A305, 1998 (abstr)
27. Gustavson SM, Chu CA, Nishizawa M, et al: Interaction of glucagon and epinephrine in the control of hepatic glucose production in the conscious dog. Am J Physiol Endocrinol Metab 284:E695-707, 2003
28. Chu CA, Sindelar DK, Neal DW, et al: Comparison of the direct and indirect effects of epinephrine on hepatic glucose production. J Clin Invest 99:1044-1056, 1997
29. Sherck SM, Shiota M, Saccomando J, et al: Pancreatic response to mild non-insulin-induced hypoglycemia does not involve extrinsic neural input. Diabetes 50:2487-2496, 2001
30. Moller N, Rizza RA, Ford GC, et al: Assessment of postabsorptive renal glucose metabolism in humans with multiple glucose tracers. Diabetes 50:747-751, 2001
31. Joseph SE, Heaton N, Potter D, et al: Renal glucose production compensates for the liver during the anhepatic phase of liver transplantation. Diabetes 49:450-456, 2000
32. Cersosimo E, Ajmal M, Naukam RJ, et al: Role of the kidney in plasma glucose regulation during hyperglycemia. Am J Physiol 272: E756-761, 1997
33. Stumvoll M, Meyer C, Perriello G, et al: Human kidney and liver gluconeogenesis: Evidence for organ substrate selectivity. Am J Physiol 274:E817-826, 1998
34. Meyer C, Stumvoll M, Dostou J, et al: Renal substrate exchange and gluconeogenesis in normal postabsorptive humans. Am J Physiol Endocrinol Metab 282:E428-434, 2002
35. Stumvoll M, Perriello G, Meyer C, et al: Role of glutamine in human carbohydrate metabolism in kidney and other tissues. Kidney Int 55:778-792, 1999
36. Brundin T, Wahren J: Renal oxygen consumption, thermogenesis, and amino acid utilization during i.v. infusion of amino acids in man. Am J Physiol 267:E648-655, 1994
37. Felig P, Owen OE, Wahren J, et al: Amino acid metabolism during prolonged starvation. J Clin Invest 48:584-594, 1969
38. Arem R: Hypoglycemia associated with renal failure. Endocrinol Metab Clin North Am 18:103-121, 1989
39. Meyer C, Stumvoll M, Nadkarni V, et al: Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. J Clin Invest 102:619-624, 1998
40. Bjorkman O, Felig P, Wahren J: The contrasting responses of splanchnic and renal glucose output to gluconeogenic substrates and to hypoglucagonemia in 60-h-fasted humans. Diabetes 29:610-616, 1980
41. Owen OE, Felig P, Morgan AP, et al: Liver and kidney metabolism during prolonged starvation. J Clin Invest 48:574-583, 1969
42. Meyer C, Dostou JM, Welle SL, et al: Role of human liver,

kidney, and skeletal muscle in postprandial glucose homeostasis. Am J Physiol Endocrinol Metab 282:E419-427, 2002

43. Meyer C, Dostou J, Nadkarni V, et al: Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. Am J Physiol 275:F915-921, 1998

44. Cersosimo E, Garlick P, Ferretti J: Regulation of splanchnic and renal substrate supply by insulin in humans. Metabolism 49:676-683, 2000

45. Woerle HJ, Popa E, Dostou J, et al: Exogenous insulin replacement in type 2 diabetes reverses excessive hepatic glucose release, but not excessive renal glucose release and impaired free fatty acid clearance. Metabolism 51:1494-1500, 2002