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Interaction of glucagon and epinephrine in the control of hepatic glucose production in the conscious dog

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Gustavson, Stephanie M., Chang An Chu, Makoto Nishizawa, Ben Farmer, Doss Neal, Ying Yang, E. Patrick Donahue, Paul Flakoll, and Alan D. Cherrington. Interaction of glucagon and epinephrine in the control of hepatic glucose production in the conscious dog. *Am J Physiol Endocrinol Metab* 284: E695–E707, 2003. First published December 27, 2002; 10.1152/ajpendo.00308.2002.—Epinephrine increases net hepatic glucose output (NHGO) mainly via increased gluconeogenesis, whereas glucagon increases NHGO mainly via increased glycogenolysis. The aim of the present study was to determine how the two hormones interact in controlling glucose production. In 18-h-fasted conscious dogs, a pancreatic clamp initially fixed insulin and glucagon at basal levels, following which one of four protocols was instituted. In G + E, glucagon (1.5 $ng \cdot kg^{-1} \cdot min^{-1}$; portally) and epinephrine (50 $ng \cdot kg^{-1} \cdot min^{-1}$; peripherally) were increased; in G, glucagon was increased alone; in E, epinephrine was increased alone; and in C, neither was increased. In G, E, and C, glucose was infused to match the hyperglycemia seen in G + E (~250 mg/dl). The areas under the curve for the increase in NHGO, after the change in C was subtracted, were as follows: G = 661 ± 185, E = 424 ± 158, G + E = 1,178 ± 57 mg/kg. Therefore, the overall effects of the two hormones on NHGO were additive. Additionally, glucagon exerted its full glycogenolytic effect, whereas epinephrine exerted its full gluconeogenic effect, such that both processes increased significantly during concurrent hormone administration.

canine; gluconeogenesis; glycogenolysis; counterregulatory hormones

GLUCAGON AND EPINEPHRINE, the two primary counterregulatory hormones, are secreted in response to physiological stresses such as hypoglycemia, exercise, and infection. The individual actions of these two hormones on glucose production have been well defined, yet it remains unclear how they interact acutely in a physiological setting to stimulate glucose production. Glucagon has been shown to have rapid effects on hepatic glucose production, with half-maximal activation occurring in ~4.5 min (19). In conscious dogs, administration of glucagon at a fourfold basal rate in the presence of a pancreatic clamp and fixed basal insulin resulted in a rapid increase (180%) in glucose production that waned with time, such that after 3 h it was increased by only 41% (7). This effect of glucagon on glucose production has been shown to result primarily from a rapid, potent, time-dependent effect on glycogenolysis and to a lesser extent from a less potent, slower effect on gluconeogenesis (7). Studies in humans have also shown that glucagon can increase glucose production in a rapid, time-dependent manner primarily by increasing glycogenolysis (8, 41).

The mild effect of glucagon on gluconeogenesis is somewhat surprising when it is considered that the hormone is known to stimulate both transcription and activation of hepatic gluconeogenic enzymes (22, 39, 49, 50). In fact, glucagon has been shown to increase hepatic gluconeogenic efficiency in vivo both acutely (67) and chronically (43), yet the contribution of the rise in gluconeogenesis to the increase in glucose production was small. This paradox may be explained by the fact that glucagon has little effect on gluconeogenic substrate mobilization from muscle or fat. Thus any enhancement of gluconeogenic flux would initially increase gluconeogenesis, but then the gluconeogenic substrate levels in blood would fall and the gluconeogenic contribution to glucose production would return toward its basal rate.

Epinephrine has also been shown to increase glucose production in a rapid, time-dependent manner, albeit with a decreased sensitivity on a molar basis compared with glucagon (6, 56, 59, 66). The effect of epinephrine on glucose production results from a stimulation of both gluconeogenesis and glycogenolysis. Chu and colleagues (10–12) showed that the former is due to the indirect action of the hormone on peripheral substrate release, whereas the latter is due to the direct action of epinephrine on the liver. Chu et al. (10) also showed that when the hormone increased gluconeogenesis, it caused a compensatory decrease in its glycogenolytic action, implying a reciprocal relationship between the two processes. Support for a reciprocal relationship between gluconeogenesis and glycogenolysis can be found in several other previous studies in both humans (33, 34, 74) and dogs (15, 18). In those experiments,

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increasing the gluconeogenic precursor supply to the liver increased gluconeogenesis but did not increase total glucose production, thereby implying a decrease in glycogenolysis. On the other hand, inhibiting glycogen breakdown has not been uniformly shown to stimulate gluconeogenesis (23, 64), perhaps because gluconeogenic precursor supply was limiting.

The interaction of glucagon and epinephrine in regulating hepatic glucose production has not been extensively characterized. Two previous studies found that administration of glucagon and epinephrine concurrently resulted in an additive increase in glucose production in the dog (21) and human (62). However, insulin and glucose levels were not controlled in those studies, making interpretation of the data difficult. In addition, glucose production was not separated into its gluconeogenic and glycogenolytic components. Thus the aim of the present study was to analyze the interaction of glucagon and epinephrine in controlling hepatic glucose production at a time when plasma insulin was basal and fixed. Specifically, we wanted to determine whether glucagon, when elevated in the presence of an epinephrine-induced increase in gluconeogenic precursor supply to the liver, would have an increased effect on gluconeogenesis and as a result a decreased effect on glycogenolysis.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were performed on 23 overnight-fasted, conscious mongrel dogs of either sex (19–26.9 kg, mean = 23.2 kg). Animals were fed 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 before the study, a laparotomy was performed under general anesthesia (15 mg/kg body wt sodium pentothal before surgery; 1.0% isoflurane as an inhalation anesthetic during surgery). In all dogs, ultrasonic flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein and a hepatic artery, as previously described (10). Silastic catheters (Dow Corning, Midline, MI) were inserted into a femoral artery, the portal vein, and the left common hepatic vein for blood sampling and into the splenic and jejunal veins for intraportal hormone delivery, as previously described (47). 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 leads were placed in subcutaneous pockets until the study day. Animals were studied only if the following criteria were met before the study: *1*) leukocyte count $<$18,000/mm^3, *2*) hematocrit $>$35%, *3*) good appetite, and *4*) normal stools. As a side note, in all dogs an ultrasonic flow probe was positioned around a renal artery, and a Silastic catheter was inserted into a renal vein. The renal glucose production data form the basis of a separate study.

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 indocyanine green dye (ICG; Sigma Chemical, St. Louis, MO) and 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) infusion.

Experimental design. Each experiment consisted of a 100-min tracer equilibration and hormone adjustment period (−140 to −40 min) followed by a 40-min control period (−40 to 0 min). During these periods, [3-^{3}H]glucose (~50 μCi prime; ~0.50 μCi/min) and ICG (0.07 mg/min) were 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-h experimental period (0–240 min) during which basal insulin was maintained. Each dog underwent one of four experimental protocols. In the G + E group (n = 6), 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 the G group (n = 6), glucagon (1.5 $ng \cdot kg^{-1} \cdot min^{-1}$; portally) alone was increased; in the E group (n = 6), epinephrine (50 $ng \cdot kg^{-1} \cdot min^{-1}$; peripherally) alone was raised; and in the C group (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 to clamp the glucose specific activity and thereby minimize errors in glucose turnover calculation. In addition, 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 min, respectively. In dogs receiving threefold basal glucagon, glucagon infusion was increased from 1.5 to 1.62, 1.74, and 1.86 at times 60, 120, and 180 min, respectively. In all dogs, mean arterial blood pressure and heart rate were determined throughout the experiment at each sampling time point by use of either a chart recorder with blood pressure transducer (Gould RS3200) or a Digi-Med Blood Pressure Analyzer (Micro-Med, Louisville, KY).

Analytical procedures. The immediate processing of the samples and the measurement of whole blood glucose, glutamine, glutamate, acetoacetate, individual amino acids (serine, threonine, glycine), and metabolites [lactate, alanine, glycerol, β-hydroxybutyrate (BOHB)] were described previously (10, 63). In addition, plasma levels of glucose, [3-^{3}H]glucose, ICG, catecholamines, insulin, glucagon, cortisol, and nonesterified fatty acids (NEFA) were measured as previously described (10, 63). C-peptide [in plasma to which 500 kallikrein inhibitor units/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%.

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Calculations. Both ICG and Transonic flow probes were used to estimate total hepatic blood flow in these studies. The net hepatic balances and net hepatic fractional extractions of the measured substrates were calculated using both Transonic-determined and ICG-determined flow. The data shown are those calculated using Transonic-determined flow, as this flow does not require an assumption about the distribution of arterial vs. portal flow. Note that the same conclusions were drawn when ICG-determined flow was used to calculate the data. Equations used were as follows

$$\text{net hepatic balance} = H \cdot HF - [(A \cdot AF) + (P \cdot PF)]$$

$$\text{net hepatic fractional extraction} = \frac{H \cdot HF - [(A \cdot AF) + (P \cdot PF)]}{[(A \cdot AF) + (P \cdot PF)]}$$

$$\text{hepatic sinusoidal level} = A \cdot (AF/HF) + P \cdot (PF/HF)$$

where A, P, and H are arterial, portal vein, and hepatic vein concentrations (blood or plasma); AF and PF are the arterial and portal vein flow (blood or plasma) measured by the Transonic flow probes; and HF (total liver flow; blood or plasma) = AF + PF. Positive numbers for net hepatic balance indicate net production, and negative numbers indicate net uptake. In some cases, uptake is presented rather than balance, and when such is the case positive values are used. Note that, because the liver is supplied by blood from both the hepatic artery and the portal vein, neither represents the true inflowing hepatic blood supply. For this reason, we calculated hepatic sinusoidal hormone levels, which provide an estimate of the average inflowing hormone concentration at the confluence of the two inputs, with the assumption that it occurs early in the sinusoid.

Tracer-determined total glucose production (R_a) and utilization (R_d) were calculated according to the isotope dilution method outlined by Wall et al. (72), as simplified by DeBodo et al. (17), and using the two-compartment model described by Mari (42) and canine parameters established by Dobbins et al. (20). Endogenous R_a was then calculated by subtracting the glucose infusion rate from the total glucose production rate. Note that endogenous glucose production represents both hepatic and renal glucose production and thus slightly overestimates hepatic glucose production.

Gluconeogenesis, as classically defined, is the synthesis and subsequent release of glucose from noncarbohydrate precursors. Carbon produced from flux through the gluconeogenic pathway does not necessarily have to be released as glucose; it can also be stored as glycogen, oxidized, or released as lactate. Therefore, there is a distinction between gluconeogenic flux to glucose 6-phosphate (G-6-*P*) (conversion of precursors to G-6-*P*, also called G-6-*P*-neogenesis) and gluconeogenesis (release of glucose derived from gluconeogenic flux). In the present studies, we estimated hepatic gluconeogenic (GNG) flux to G-6-*P*, net hepatic GNG flux, and net hepatic glycogenolytic (GLY) flux.

Hepatic GNG flux to G-6-*P* was obtained by summing net hepatic uptake rates of the gluconeogenic precursors (alanine, serine, glycine, threonine, glutamine, glutamate, glycerol, lactate, pyruvate) and then dividing by two to transform the data into glucose equivalents (by accounting for incorporation of three-carbon precursor molecules into six-carbon glucose molecules). Net hepatic pyruvate uptake was assumed to be 10% of net hepatic lactate uptake (71). When net hepatic output of any precursor occurred, rather than uptake, the precursor was considered to be a product of the liver, and thus net uptake was set to zero. However, note that the net hepatic balance data of the precursors represent the entire database, regardless of net output or net uptake.

Net hepatic GNG flux was determined by subtracting the summed net hepatic output rates (when such occurred) of the substrates noted above (in glucose equivalents) and glucose oxidation from the GNG flux to G-6-*P*. A positive number represents net gluconeogenic flux to G-6-*P,* whereas a negative number indicates net glycolytic flux from G-6-*P*. Glucose oxidation was assumed to be 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ throughout each experiment, similar to the basal period of earlier studies in 18-h-fasted (28) and 24-h-fasted (0.3 ± 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$; Moore MC, Pagliassotti MJ, Swift LL, Asher J, Murrell J, Neal D, and Cherrington AD, unpublished observations) conscious dogs. Although use of this value may slightly overestimate or underestimate the true glucose oxidation rate, it is unlikely to differ by >0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ from the actual oxidation rate. Our earlier studies (60) showed that hyperglycemia (in the presence of euinsulinemia) did not appreciably change the hepatic glucose oxidation rate (0.4 ± 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$). It also seems unlikely that glucagon and epinephrine would change hepatic glucose oxidation significantly. Although both have been shown to inhibit pyruvate dehydrogenase, and thus pyruvate oxidation (24, 54, 55), the basal oxidation rate is so low that any effect would have been difficult, if not impossible, to detect.

Net hepatic GLY flux was determined by subtracting net hepatic GNG flux from net hepatic glucose balance (NHGB). A positive number therefore represents net glycogen breakdown, whereas a negative number indicates net glycogen synthesis.

Ideally, GNG flux to G-6-*P* would be calculated using unidirectional hepatic uptake rates for each substrate, but this would be difficult, as it would require the simultaneous use of multiple stable isotopes that could themselves induce a mild perturbation of the metabolic state. Therefore, net hepatic balance was used instead, necessitating consideration of the limits of this approach. There is little or no net production of gluconeogenic amino acids or glycerol by the liver, so in their case the compromise is of little consequence (26, 46). However, such is not the case for lactate. Our estimate of the rate of GNG flux to G-6-*P* will be quantitatively accurate only if we assume that lactate flux is unidirectional at a given moment (i.e., either into or out of the liver). In a given cell, this does not seem like an unreasonable assumption in light of the reciprocal control of gluconeogenesis or glycogenolysis (50). Jungermann and Katz (35) and Radziuk and Pye (53) have suggested, however, that there is spatial separation of metabolic pathways. Specifically, gluconeogenic periportal hepatocytes primarily consume lactate and other noncarbohydrate precursors for the synthesis of glucose and glycogen, whereas glycolytic perivenous hepatocytes predominantly consume plasma glucose, which can then be incorporated into glycogen, oxidized, or released as lactate or other glycolytic substrates (35, 53). Therefore, it is possible that, under normal nutritional conditions, hepatic GNG and GLY flux occur in a net sense simultaneously with lactate output or uptake occurring in different cells. To the extent that flux occurs in both directions simultaneously, use of net hepatic balance will cause an underestimation of GNG flux to G-6-*P*. Note that net hepatic GNG flux and net hepatic GLY flux can be calculated accurately without concern for the assumptions related to whether or not simultaneous GNG and GLY substrate flux occur.

The approach we used to estimate GNG flux to G-6-*P* is based on several assumptions. First, it is assumed that there is minimal net contribution of gluconeogenic precursors from intrahepatic proteolysis and lipolysis. To the extent that there is a small contribution of intrahepatic gluconeogenic

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precursors, we would tend to underestimate GNG flux (and overestimate GLY flux). However, we have found that, in the normal dog, there are negligible hepatic triglyceride stores after an overnight fast (Moore MC, Pagliassotti MJ, Swift LL, Asher J, Murrell J, Neal D, and Cherrington AD, unpublished observations). This observation of low hepatic triglyceride stores in the dog was supported by another group (70). Furthermore, we recently estimated that intrahepatic proteolysis after an overnight fast in the dog was only 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$, thus contributing minimally to gluconeogenic flux (26). We also showed that, in the 36-h-fasted dog, alanine specific activity exiting the liver was identical to that entering the liver under basal hormonal conditions, suggesting that there was minimal intrahepatic proteolysis (65). Although glucagon, cAMP, and epinephrine have been shown to stimulate hepatic proteolysis in vitro, pharmacological levels were required for a modest effect (45, 46, 48, 57, 61, 75), whereas physiological levels similar to those in the present study stimulated proteolysis only minimally (<0.5%) (30) or not at all (48). A second assumption of the method is that all of the gluconeogenic carbon taken up in a net sense is converted to G-6-*P*. We verified this assumption in a recent study which showed that GNG flux measured directly was actually larger than the estimate obtained using the current method, presumably due to the addition of intrahepatic amino acid precursors (26). A third assumption is that transient variations in the intrahepatic pool of gluconeogenic substrates have minimal impact on our estimates of GNG flux.

The area under the curve (AUC) for hepatic GNG flux to G-6-*P*, net hepatic GNG flux, and net hepatic GLY flux in each group was calculated for the entire experimental period (4 h) by use of the trapezoidal rule. The AUC was calculated using change from basal data points, thus accounting for any baseline differences among groups. The mean AUC of the control group was then subtracted from that of each individual dog in every group. The mean ± SE for the ΔAUC for each of the three experimental groups was then reported.

Statistical analysis. Data are expressed as means ± SE. Statistical comparisons were made by one- and two-way analysis of variance (ANOVA) with repeated-measures design (except for the blood pressure and heart rate data: paired *t*-test) run on Sigma Stat (SPSS Science, Chicago, IL). Analysis of AUC data was made with one-way ANOVA. Post hoc analysis was performed with Tukey's test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Glucose and hormone levels. In all four groups, plasma glucose levels rose from ~110 to ~250 mg/dl (Table 1). To achieve similar glucose levels in all groups, different glucose infusion rates (GIR) were required, as depicted in Table 1. The plasma insulin levels remained essentially unchanged and basal and were not significantly different from group to group (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 and hepatic sinusoidal 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 and Fig. 1). Arterial and hepatic sinusoidal 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 and Fig. 1). Arterial cortisol levels as well as arterial and portal norepinephrine levels remained basal in all groups throughout the studies (data not shown).

Arterial blood pressure and heart rate. Mean arterial blood pressure (mmHg) was initially similar in all groups (basal period: C = 106 ± 5, G = 121 ± 11, E = 112 ± 9, G + E = 129 ± 9) and remained stable in all but the E group, in which it fell modestly (average of experimental period: C = 106 ± 5, G = 121 ± 8, E = 96 ± 11, G + E = 130 ± 11, $P < 0.05$ for the change in E; paired *t*-test). As expected, heart rate rose modestly in both E and G + E ($P < 0.05$; paired *t*-test) as a result of epinephrine administration (C = 96 ± 12 to 94 ± 6, G = 92 ± 17 to 82 ± 11, E = 104 ± 13 to 134 ± 8, G + E = 70 ± 9 to 95 ± 9).

Glucose metabolism. In all groups, basal NHGB ($mg \cdot kg^{-1} \cdot min^{-1}$) was similar (C = 1.2 ± 0.2, G = 1.7 ± 0.3, E = 1.8 ± 0.3, G + E = 1.4 ± 0.2; Fig. 2). In response to hyperglycemia (C), NHGB changed from output to uptake (−2.5 ± 0.3 at 240 min). In response to glucagon (G), NHGB rose to 4.6 ± 0.8 at 15 min and waned with time (0.5 ± 0.8 at 240 min). The effect of glucagon per se is represented in the *inset* to Fig. 2 as the difference between the changes in G and C. In response to epinephrine (E), NHGB rose (3.3 ± 0.9 at 15 min) but also waned with time, falling to a rate significantly lower than basal (0.0 ± 1.0 at 240 min). The effect of epinephrine per se is represented in the *inset* of Fig. 2 as the difference between the changes in E and C. Finally, in the presence of both hormones (G + E), NHGB rose to 7.3 ± 1.0 at 15 min, which was greater than with either individual hormone. Once again, the response waned with time (2.0 ± 0.5 at 240 min). The data in the *inset* of Fig. 2 indicate that the effects of glucagon and epinephrine on net hepatic glucose production (ΔAUC) were additive. Changes in tracer-determined endogenous glucose R_a paralleled the changes in NHGB (Table 2).

Tracer-determined, whole body glucose R_d ($mg \cdot kg^{-1} \cdot min^{-1}$; Table 2) increased markedly in C (2.3 ± 0.2 to 6.0 ± 0.5 at 240 min). In G and E, glucose R_d rose less than in the control group (2.7 ± 0.2 to 4.2 ± 1.0 with G and 2.9 ± 0.2 to 4.0 ± 0.9 with E at 240 min). Finally, when both hormones were given together, glucose R_d did not rise significantly (2.8 ± 0.4 to 3.4 ± 0.8 at 240 min).

Lactate: arterial levels and net hepatic balance. In the control group, arterial blood lactate levels rose modestly due to an increase in net hepatic lactate output during hyperglycemia (Fig. 3). When glucagon was increased, the arterial blood lactate level rose as in the control group, also due to an increase in net hepatic lactate production. However, with glucagon, the rise in net hepatic lactate output and the lactate level occurred more quickly, presumably resulting from the hormone's effect on glycogenolysis. When epinephrine was increased, arterial lactate levels rose to a markedly greater extent than in C or G despite the fact that

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Table 1. *Arterial plasma glucose, GIR, arterial plasma insulin, hepatic sinusoidal plasma insulin, and arterial plasma glucagon and epinephrine*

Time, min	−40	0	15	30	60	90	120	180	240
Arterial glucose, mg/dl									
C	117 ± 6	115 ± 6	142 ± 4	172 ± 6	234 ± 4	241 ± 3	249 ± 1	250 ± 3	247 ± 3
G	110 ± 3	108 ± 3	139 ± 7	186 ± 7	228 ± 13	243 ± 15	250 ± 14	257 ± 13	251 ± 13
E	112 ± 4	112 ± 4	133 ± 3	173 ± 6	222 ± 13	235 ± 16	246 ± 11	240 ± 10	237 ± 10
G + E	106 ± 4	107 ± 3	136 ± 3	178 ± 9	221 ± 14	239 ± 15	248 ± 16	251 ± 18	246 ± 20
GIR, $mg \cdot kg^{-1} \cdot min^{-1}$									
C		4.5 ± 0.7	4.1 ± 0.9	7.1 ± 0.9	6.9 ± 0.8	4.6 ± 0.3	5.1 ± 0.3	5.0 ± 0.3	6.3 ± 1.3
G		0 ± 0	2.9 ± 0.4	3.6 ± 1.5	3.1 ± 1.3	3.9 ± 1.3	2.9 ± 1.0	2.9 ± 1.1	3.0 ± 1.2
E		0 ± 0	3.5 ± 1.0	4.7 ± 0.8	4.1 ± 0.9	3.4 ± 0.8	2.5 ± 0.6	2.2 ± 0.7	2.4 ± 0.6
G + E		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arterial insulin, μU/ml									
C	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	3 ± 1	4 ± 1	5 ± 1	5 ± 1
G	5 ± 1	5 ± 1	6 ± 1	5 ± 1	6 ± 1	6 ± 1	5 ± 1	7 ± 2	6 ± 1
E	5 ± 1	5 ± 1	7 ± 1	7 ± 2	5 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1
G + E	4 ± 1	4 ± 1	5 ± 1	4 ± 1	4 ± 1	5 ± 1	6 ± 1	6 ± 1	5 ± 1
Sinusoidal insulin, μU/ml									
C	15 ± 3	17 ± 5	15 ± 4	16 ± 5	13 ± 3	14 ± 3	15 ± 2	18 ± 3	18 ± 5
G	16 ± 3	15 ± 3	17 ± 4	16 ± 3	18 ± 3	19 ± 3	17 ± 3	18 ± 3	21 ± 3
E	18 ± 2	14 ± 2	16 ± 3	17 ± 2	18 ± 3	19 ± 3	17 ± 2	21 ± 3	20 ± 3
G + E	15 ± 3	13 ± 2	15 ± 2	14 ± 2	12 ± 1	13 ± 2	17 ± 3	13 ± 2	14 ± 2
Arterial glucagon, pg/ml									
C	44 ± 6	44 ± 5	37 ± 4	39 ± 5	39 ± 4	39 ± 3	42 ± 6	37 ± 6	39 ± 5
G	51 ± 7	49 ± 8	75 ± 10	81 ± 9	80 ± 9	78 ± 10	73 ± 10	72 ± 10	73 ± 10
E	43 ± 6	39 ± 3	41 ± 3	41 ± 2	44 ± 5	40 ± 4	37 ± 3	34 ± 1	38 ± 2
G + E	46 ± 4	41 ± 3	76 ± 9	81 ± 10	78 ± 9	78 ± 9	77 ± 8	77 ± 8	77 ± 9
Arterial epinephrine, pg/ml									
C	175 ± 63	214 ± 46	188 ± 77	148 ± 61	201 ± 44	161 ± 42	172 ± 74	252 ± 75	172 ± 41
G	145 ± 43	174 ± 40	184 ± 44	181 ± 33	154 ± 35	172 ± 29	191 ± 54	172 ± 38	158 ± 45
E	164 ± 34	143 ± 43	1245 ± 123	1232 ± 209	1089 ± 178	1007 ± 191	1085 ± 141	1074 ± 124	1146 ± 264
G + E	189 ± 39	200 ± 53	1102 ± 240	940 ± 128	1070 ± 74	936 ± 187	1309 ± 418	832 ± 139	1126 ± 119

Data are means ± SE. For matched hyperglycemia (C), $n = 5$; for glucagon alone (G), epinephrine alone (E), and G + E, $n = 6$. GIR, glucose infusion rate. For arterial plasma glucose, there was no significant difference among groups, and all groups increased from basal ($P < 0.05$). For GIR, $P < 0.05$ for C vs. G, E, and G + E, and for G + E vs. C, G, and E. For arterial plasma insulin, there was no significant difference among groups, although in E and G + E it increased slightly from basal ($P < 0.05$). For sinusoidal plasma insulin, there was no difference among groups, and no group changed significantly from basal. For arterial glucagon, $P < 0.05$ for C vs. G and G + E; and E vs. G and G + E. G and G + E changed significantly from basal ($P < 0.05$), whereas C and E did not. For arterial epinephrine, $P < 0.05$ for C vs. E and G + E; and G vs. E and G + E. E and G + E changed significantly from basal ($P < 0.05$), whereas C and G did not.

net hepatic output essentially ceased within 30 min. This indicates that the catecholamine stimulated the net release of lactate from nonhepatic tissues (most likely muscle). Finally, when both hormones were increased concurrently, there was a brief increase in net hepatic lactate output and a resulting rise in the blood lactate level, followed by a fall in net hepatic lactate output to zero and a continued rise in the lactate level to almost 2.5 mmol/l.

Glycerol, NEFA, and ketones: arterial levels, net hepatic balance, and net hepatic fractional extraction. In both the hyperglycemic control protocol and the glucagon protocol, arterial glycerol levels and net hepatic glycerol uptake drifted down (significantly in G, nonsignificantly in C; Table 3). Epinephrine caused a rise in both arterial glycerol levels and net hepatic glycerol uptake, both of which waned with time. Finally, the combination of glucagon and epinephrine resulted in changes that were similar to those seen with epinephrine alone. Net hepatic glycerol fractional extraction did not change over time in any group and was not different among the groups (data not shown).

The NEFA data closely resemble the glycerol data. In the C and G groups, both arterial NEFA levels and net hepatic NEFA uptake fell significantly (Table 3). In the E and G + E groups, there was an early rise in both arterial NEFA levels and net hepatic NEFA uptake, both of which waned with time. Notably, when both hormones were administered concurrently, NEFA levels and uptake tended to remain elevated for a more prolonged period before falling. Net hepatic NEFA fractional extraction did not change in any group and was not different among the groups (data not shown).

Ketone (BOHB and acetoacetate) metabolism tended to mirror changes in NEFA, although statistical significance was not achieved. Arterial blood ketone levels and net hepatic production tended to fall in both C and G (Table 3). In E, blood ketone levels tended to rise and then fall, as did net hepatic production. Finally, in G + E, ketone levels and net hepatic production also tended to rise and fall, although the increase appeared more sustained.

Alanine: arterial levels, net hepatic uptake, and net hepatic fractional extraction. In the hyperglycemic control group, arterial alanine levels rose, net hepatic alanine uptake did not change, and net hepatic fractional extraction of alanine tended to fall (Table 4). In

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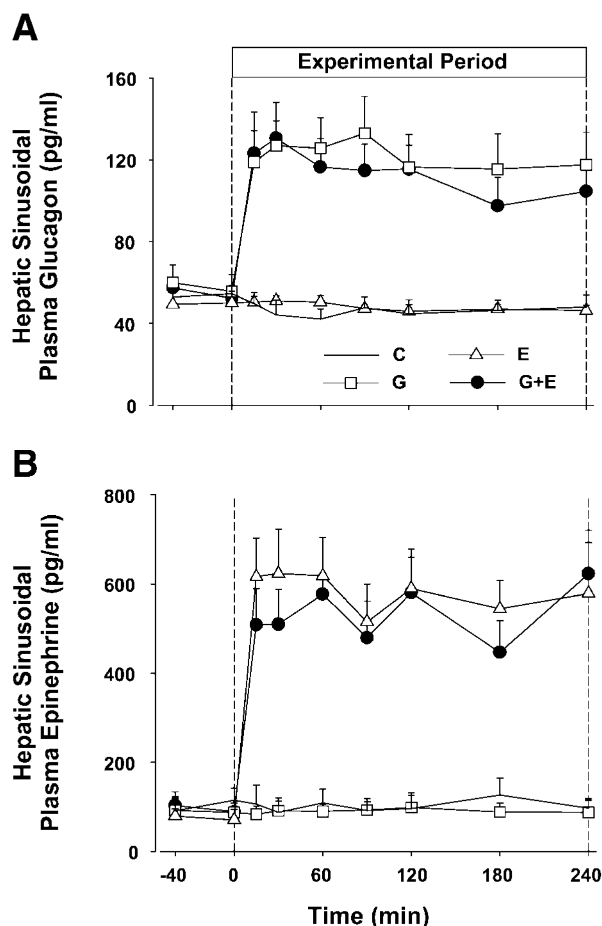

Fig. 1. Hepatic sinusoidal plasma glucagon (*A*) and epinephrine levels (*B*) in control (−40 to 0 min) and experimental (0–240 min) periods in the hyperglycemic control (C), glucagon-alone (G), epinephrine-alone (E), and the 2 hormones combined (G + E) 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated measures; $n = 5$ for C, $n = 6$ each for G, E, and G + E. For sinusoidal glucagon, $P < 0.05$ for C vs. G and G + E and for E vs. G and G + E. G and G + E changed significantly from basal ($P < 0.05$), whereas C and E did not. For sinusoidal epinephrine, $P < 0.05$ for C vs. E and G + E and for G vs. E and G + E. E and G + E changed significantly from basal ($P < 0.05$), whereas C and G did not.

the epinephrine infusion group, both the arterial level and net hepatic uptake of alanine increased, whereas net hepatic fractional extraction was sustained. In the two groups involving glucagon infusion, the arterial alanine levels did not change, but net hepatic alanine uptake increased and net hepatic alanine fractional extraction tended to increase. Although only the alanine data are portrayed, as it is the most important gluconeogenic amino acid, the calculations to determine GNG and GLY flux incorporated the net hepatic balance of the other gluconeogenic amino acids as well (serine, threonine, glycine, glutamine, and glutamate).

Gluconeogenesis and glycogenolysis. In response to hyperglycemia (Fig. 4), hepatic GNG flux to G-6-*P* ($mg \cdot kg^{-1} \cdot min^{-1}$) did not change, whereas net hepatic GNG flux fell (−0.5 ± 0.2 to −1.3 ± 0.3 at 240 min, $P < 0.05$). Net hepatic GLY flux ($mg \cdot kg^{-1} \cdot min^{-1}$) also fell when hyperglycemia occurred (1.6 ± 0.3 to −1.4 ± 0.1 at 240 min, $P < 0.05$). In response to glucagon (Fig. 4), GNG flux to G-6-*P* did not change significantly, whereas net hepatic GNG flux fell quickly (by 15 min; $P < 0.05$) and remained modestly suppressed relative to its basal value. Net hepatic GLY flux increased initially (1.9 ± 0.4 to 5.9 ± 1.0 at 15 min) and then waned with time (1.0 ± 0.7 at 240 min). In response to epinephrine (Fig. 5), hepatic GNG flux to G-6-*P* almost tripled by 240 min ($P < 0.05$). Net hepatic GNG flux also increased significantly (−0.8 ± 0.4 to 0.7 ± 0.6 at 240 min, $P < 0.05$). In contrast, there was a nonsignificant rise in net hepatic GLY flux (2.5 ± 0.6 to 3.2 ± 1.2 at 15 min) that waned with time, eventually reaching a rate significantly below basal (−1.0 ± 0.8 at 240 min). Finally, in response to both hormones (Fig. 6), hepatic GNG flux to G-6-*P* increased significantly, albeit to a slightly lesser extent than with epinephrine alone. Net hepatic GNG flux also increased in a similar manner ($P < 0.05$). In contrast, net hepatic GLY flux increased significantly (1.7 ± 0.6 to 8.1 ± 1.6 at 15 min) and to a

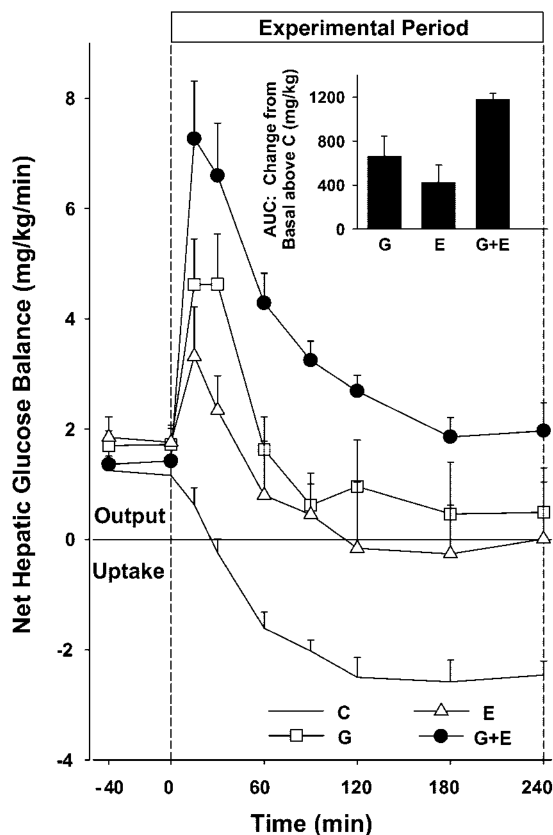

Fig. 2. Net hepatic glucose balance in control (−40 to 0 min) and experimental (0–240 min) periods in C, G, E, and G + E 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 1- and 2-way ANOVA with repeated measures; $n = 5$ for C, $n = 6$ each for G, E, and G + E. $P < 0.05$ for C vs. G, E and G + E, G vs. C and G + E, and E vs. C and G + E. All groups changed from basal ($P < 0.05$; C and E fell significantly by the end of the study, and G and G + E rose significantly and then returned to basal levels). *Inset*: area under the curves (AUC) of net hepatic glucose balance (change from basal after subtracting change from basal of C, over 4 h). $P < 0.05$ for G + E vs. G and E.

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Table 2. *Tracer-determined whole body glucose R_a and R_d*

Time, min	−40	0	30	60	90	120	180	240
Endogenous R_a								
C	2.5 ± 0.2	2.1 ± 0.2	1.2 ± 0.6	0.7 ± 0.3	1.9 ± 0.5	1.0 ± 0.4	0.9 ± 0.3	−0.1 ± 0.6
G	2.7 ± 0.2	2.6 ± 0.2	5.0 ± 1.3	4.3 ± 0.9	2.2 ± 0.8	2.3 ± 0.5	1.8 ± 0.5	1.1 ± 0.6
E	3.1 ± 0.2	2.7 ± 0.2	3.0 ± 0.6	2.6 ± 0.3	2.2 ± 0.4	2.5 ± 0.4	1.9 ± 0.6	1.6 ± 0.7
G + E	2.6 ± 0.3	2.8 ± 0.5	7.4 ± 0.8	6.5 ± 0.5	4.9 ± 0.3	3.9 ± 0.4	3.3 ± 0.3	3.3 ± 0.7
R_d								
C	2.5 ± 0.2	2.2 ± 0.1	3.5 ± 0.4	4.7 ± 0.5	5.5 ± 0.4	6.2 ± 0.5	6.0 ± 0.3	6.0 ± 0.5
G	2.8 ± 0.3	2.6 ± 0.2	3.3 ± 0.4	4.0 ± 0.2	4.6 ± 0.5	5.1 ± 0.5	4.9 ± 0.9	4.2 ± 1.0
E	3.1 ± 0.3	2.7 ± 0.2	3.4 ± 0.4	3.6 ± 0.3	4.1 ± 0.4	4.9 ± 0.6	4.4 ± 0.8	4.0 ± 0.9
G + E	2.8 ± 0.3	2.8 ± 0.4	2.8 ± 0.5	3.2 ± 0.5	3.5 ± 0.6	3.4 ± 0.6	3.7 ± 0.4	3.4 ± 0.8

Data are means ± SE in $mg \cdot kg^{-1} \cdot min^{-1}$. For C, $n = 5$; for G, E, and G + E, $n = 6$. R_a, glucose production; R_d, glucose utilization. For endogenous R_a, $P < 0.05$ for G vs. C, and for G + E vs. C, G, E. C decreased from basal ($P < 0.05$), G and G + E increased ($P < 0.05$) and then waned with time, whereas E did not change significantly. For R_d, $P < 0.05$ for G + E vs. C. All groups increased significantly from basal ($P < 0.05$) except G + E.

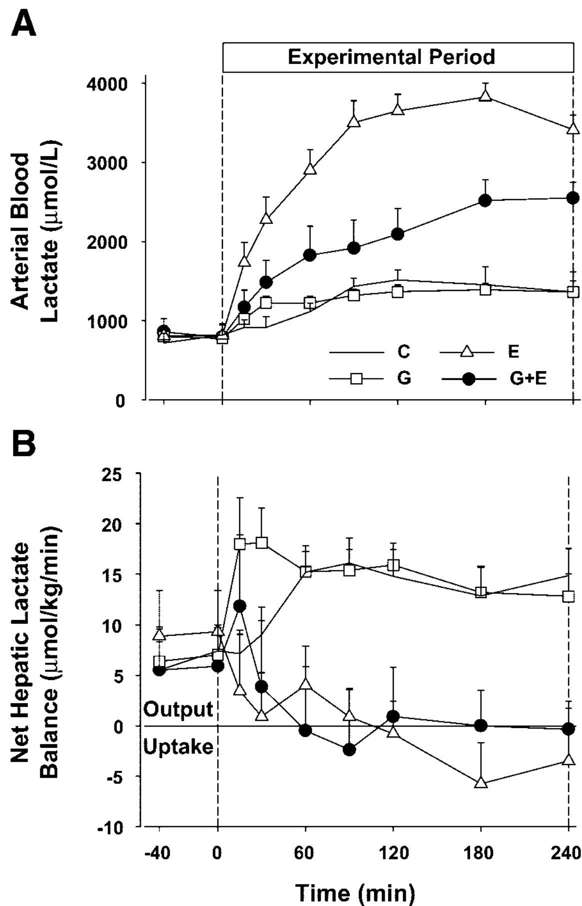

Fig. 3. Arterial blood lactate levels (*A*) and net hepatic lactate balance (*B*) in control (−40 to 0 min) and experimental (0–240 min) periods in C, G, E, and G + E 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated measures; $n = 5$ for C, $n = 6$ each for G, E, and G + E. For arterial lactate levels, $P < 0.05$ for E vs. C, G, and G + E. Although there was no overall significant difference of G + E vs. C and G, the last 2 time points were significantly different ($P < 0.05$). All groups increased significantly from basal ($P < 0.05$). For net hepatic lactate balance, $P < 0.05$ for G vs. E. All groups changed significantly from basal ($P < 0.05$; C and G rose significantly, whereas E and G + E fell significantly).

greater extent than with either hormone alone, after which it waned with time (1.5 ± 0.6 at 240 min).

The ΔAUC revealed that glucagon and epinephrine do not have a synergistic effect on hepatic GNG flux to G-6-*P* or net hepatic GNG flux (Fig. 7). In fact, their effects on both parameters, as well as on net hepatic GLY flux, appear to be additive (Fig. 7). Whereas independently each could only increase one process significantly over the 4-h period, together they could simultaneously augment both gluconeogenesis and glycogenolysis.

DISCUSSION

The aim of the present study was to determine whether epinephrine could modify the action of glucagon on hepatic glucose production. We hypothesized that total hepatic production would be additive in the presence of both hormones but that glucagon's effect on gluconeogenesis would be augmented whereas its effect on glycogenolysis would be inhibited. The hormones were indeed found to have additive effects on hepatic glucose production regardless of the technique used to assess the process (NHGB or tracer-determined endogenous R_a). The present study confirmed previous findings that, over a 4-h period, glucagon's action is primarily glycogenolytic whereas epinephrine's action is primarily gluconeogenic. Contrary to our hypothesis, however, the results showed no synergistic effect of the two hormones on gluconeogenesis. Likewise, the glycogenolytic response to the two hormones was not less than the sum of their individual responses. In short, epinephrine did not alter the action of glucagon on hepatic glucose production; instead, the effects of the two hormones were additive, such that a simultaneous rise in both augmented both gluconeogenesis and glycogenolysis markedly.

These studies looked at the effects of physiological increments in glucagon and epinephrine on glucose production by the liver in the absence of changes in insulin and in the presence of matched hyperglycemia. The glucagon levels achieved were approximately one-half those needed for the hormone's maximal effect on glucose production (67). The epinephrine levels were such that they had a small but significant effect on

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Table 3. *Arterial blood levels of glycerol and ketones and arterial plasma levels of NEFA in addition to NH glycerol U, NH NEFA U, and NH ketone P*

Time, min	−40	0	15	30	60	90	120	180	240
Arterial glycerol, μmol/l									
C	59 ± 2	47 ± 4	39 ± 4	42 ± 6	43 ± 6	37 ± 6	35 ± 12	31 ± 4	37 ± 8
G	102 ± 15	88 ± 11	78 ± 14	66 ± 11	66 ± 10	63 ± 9	57 ± 11	68 ± 12	65 ± 15
E	75 ± 8	67 ± 11	123 ± 21	107 ± 21	87 ± 15	85 ± 19	86 ± 19	94 ± 21	72 ± 13
G + E	65 ± 13	64 ± 5	114 ± 20	110 ± 20	106 ± 16	96 ± 14	89 ± 17	80 ± 14	71 ± 9
Arterial NEFA, μmol/l									
C	721 ± 95	563 ± 81	524 ± 108	529 ± 81	433 ± 81	355 ± 35	297 ± 38	275 ± 28	311 ± 50
G	902 ± 141	758 ± 100	733 ± 88	573 ± 86	512 ± 87	443 ± 60	433 ± 73	438 ± 76	439 ± 80
E	686 ± 60	625 ± 72	892 ± 95	888 ± 124	601 ± 81	453 ± 47	428 ± 70	382 ± 49	364 ± 41
G + E	557 ± 99	613 ± 65	1006 ± 190	989 ± 207	972 ± 196	866 ± 183	691 ± 117	480 ± 60	470 ± 51
Arterial ketones, μmol/l									
C	107 ± 22	101 ± 21	76 ± 19	86 ± 20	80 ± 17	70 ± 19	67 ± 16	68 ± 16	77 ± 22
G	109 ± 14	105 ± 10	97 ± 12	102 ± 10	92 ± 13	91 ± 10	96 ± 12	95 ± 11	93 ± 14
E	67 ± 12	73 ± 16	97 ± 27	89 ± 13	62 ± 13	66 ± 8	55 ± 12	63 ± 10	55 ± 10
G + E	72 ± 21	61 ± 17	95 ± 21	79 ± 21	83 ± 16	77 ± 21	66 ± 16	62 ± 14	87 ± 14
NH glycerol U, $\mu mol \cdot kg^{-1} \cdot min^{-1}$									
C	1.27 ± 0.16	0.90 ± 0.07	0.81 ± 0.17	0.86 ± 0.23	0.94 ± 0.26	0.66 ± 0.17	0.55 ± 0.19	0.65 ± 0.18	0.87 ± 0.23
G	2.08 ± 0.40	1.80 ± 0.22	1.70 ± 0.32	1.42 ± 0.24	1.53 ± 0.31	1.32 ± 0.26	1.16 ± 0.37	1.42 ± 0.36	1.36 ± 0.44
E	1.25 ± 0.24	1.05 ± 0.14	2.36 ± 0.52	2.02 ± 0.49	1.46 ± 0.37	1.48 ± 0.53	1.36 ± 0.37	1.75 ± 0.69	1.36 ± 0.52
G + E	1.02 ± 0.25	0.97 ± 0.13	1.68 ± 0.30	1.76 ± 0.42	1.72 ± 0.32	1.49 ± 0.29	1.04 ± 0.28	1.26 ± 0.29	1.31 ± 0.17
NH NEFA U, $\mu mol \cdot kg^{-1} \cdot min^{-1}$									
C	2.41 ± 0.39	1.57 ± 0.31	1.88 ± 0.44	1.79 ± 0.42	1.45 ± 0.49	1.18 ± 0.22	1.00 ± 0.20	0.89 ± 0.22	1.16 ± 0.31
G	3.41 ± 0.60	2.76 ± 0.39	3.33 ± 0.68	2.58 ± 0.58	2.20 ± 0.73	1.69 ± 0.39	1.77 ± 0.46	1.91 ± 0.56	1.24 ± 0.20
E	2.40 ± 0.22	2.27 ± 0.34	3.68 ± 0.67	3.55 ± 0.99	2.18 ± 0.67	1.51 ± 0.29	1.31 ± 0.41	1.09 ± 0.27	1.25 ± 0.39
G + E	1.43 ± 0.52	1.84 ± 0.39	3.03 ± 0.67	3.19 ± 0.94	3.19 ± 0.84	2.55 ± 0.89	2.00 ± 0.49	1.46 ± 0.33	1.31 ± 0.26
NH ketone P, $\mu mol \cdot kg^{-1} \cdot min^{-1}$									
C	1.36 ± 0.43	1.28 ± 0.38	1.02 ± 0.83	1.51 ± 0.61	0.41 ± 0.66	0.75 ± 0.43	0.75 ± 0.48	0.98 ± 0.42	0.90 ± 0.48
G	1.03 ± 0.31	1.61 ± 0.74	0.43 ± 0.27	0.67 ± 0.50	0.75 ± 0.45	1.00 ± 0.57	0.17 ± 0.22	0.63 ± 0.48	0.21 ± 0.43
E	1.25 ± 0.27	1.09 ± 0.19	2.63 ± 0.76	1.58 ± 0.25	0.66 ± 0.42	0.10 ± 0.54	0.05 ± 0.61	0.39 ± 0.35	0.54 ± 0.54
G + E	1.09 ± 0.56	1.70 ± 0.22	2.24 ± 0.99	2.12 ± 0.81	1.44 ± 0.93	1.82 ± 0.67	1.18 ± 0.62	1.21 ± 0.50	0.97 ± 0.44

Data are means ± SE. For glycerol and nonesterified fatty acids (NEFA) parameters: $n = 5$ for C; $n = 6$ for G, E, and G + E. For ketone parameters: $n = 5$ for G and C; $n = 4$ for E and G + E. NH, net hepatic; U, uptake; P, production. For arterial glycerol, $P < 0.05$ for C vs. E and G + E. C did not change significantly, G fell significantly ($P < 0.05$), whereas E and G + E rose significantly ($P < 0.05$) and then returned to basal levels. For arterial NEFA, $P < 0.05$ for C vs. G + E. Both C and G decreased from basal ($P < 0.05$), whereas both E and G + E increased ($P < 0.05$) and then waned. For arterial ketones, there was no significant difference among groups, and only C changed from basal ($P < 0.05$). For NH glycerol U, there was no significant difference among groups. C and G + E did not change significantly, G fell significantly ($P < 0.05$), and E rose significantly ($P < 0.05$) and then returned to basal levels. For NH NEFA U, there was no significant difference among groups. However, C and G fell from basal ($P < 0.05$), whereas G + E increased ($P < 0.05$) and then waned. Although E also tended to rise and wane, these changes were not significant. Finally, for NH ketone P, there was no significant difference among groups, and no group changed significantly from basal.

Table 4. *Arterial blood levels, net hepatic uptake, and net hepatic fractional extraction of alanine*

Time, min	−40	0	15	30	60	90	120	180	240
Arterial alanine, μmol/l									
C	380 ± 36	380 ± 30	383 ± 38	378 ± 30	449 ± 35	493 ± 39	533 ± 38	578 ± 66	606 ± 70
G	385 ± 56	398 ± 63	405 ± 60	430 ± 69	422 ± 65	406 ± 57	407 ± 59	394 ± 48	390 ± 49
E	381 ± 49	377 ± 48	379 ± 47	412 ± 46	461 ± 53	510 ± 49	534 ± 52	549 ± 50	557 ± 44
G + E	425 ± 64	410 ± 63	405 ± 73	404 ± 84	393 ± 88	381 ± 89	376 ± 79	399 ± 58	391 ± 50
NH alanine U, $\mu mol \cdot kg^{-1} \cdot min^{-1}$									
C	2.34 ± 0.24	2.27 ± 0.51	2.53 ± 0.39	3.84 ± 1.30	3.29 ± 0.61	2.88 ± 0.39	3.10 ± 0.61	2.71 ± 0.71	2.91 ± 0.55
G	2.93 ± 0.62	2.68 ± 0.54	2.70 ± 0.48	2.94 ± 0.47	3.72 ± 0.53	3.77 ± 0.34	4.04 ± 0.44	3.70 ± 0.59	4.35 ± 0.61
E	2.21 ± 0.18	2.79 ± 0.36	3.07 ± 0.54	3.52 ± 0.61	3.10 ± 0.63	3.64 ± 0.64	4.34 ± 0.63	4.64 ± 0.50	4.27 ± 0.42
G + E	2.49 ± 0.40	2.28 ± 0.38	2.35 ± 0.65	2.64 ± 0.53	2.87 ± 0.51	2.99 ± 0.32	3.46 ± 0.17	3.70 ± 0.34	4.01 ± 0.44
NH alanine fractional extraction									
C	0.27 ± 0.06	0.24 ± 0.06	0.26 ± 0.07	0.29 ± 0.04	0.25 ± 0.03	0.20 ± 0.01	0.20 ± 0.03	0.16 ± 0.03	0.18 ± 0.03
G	0.24 ± 0.03	0.22 ± 0.02	0.24 ± 0.04	0.24 ± 0.04	0.31 ± 0.05	0.32 ± 0.05	0.34 ± 0.05	0.33 ± 0.05	0.36 ± 0.05
E	0.22 ± 0.03	0.27 ± 0.03	0.27 ± 0.06	0.29 ± 0.06	0.23 ± 0.04	0.24 ± 0.04	0.29 ± 0.04	0.29 ± 0.01	0.25 ± 0.02
G + E	0.24 ± 0.04	0.24 ± 0.04	0.23 ± 0.07	0.27 ± 0.06	0.31 ± 0.07	0.32 ± 0.06	0.34 ± 0.06	0.33 ± 0.04	0.33 ± 0.03

Data are means ± SE. For C, $n = 5$; for G, E, and G + E, $n = 6$. For arterial alanine, there was no significant difference among groups. However, C and E increased significantly ($P < 0.05$), whereas G and G + E did not change. For NH alanine U, there was no significant difference among groups, and all groups except C increased significantly ($P < 0.05$). For NH fractional extraction of alanine, there was no significant difference among groups. The NH fractional extraction of alanine did not change significantly in C, E, or G + E but increased significantly in G ($P < 0.05$).

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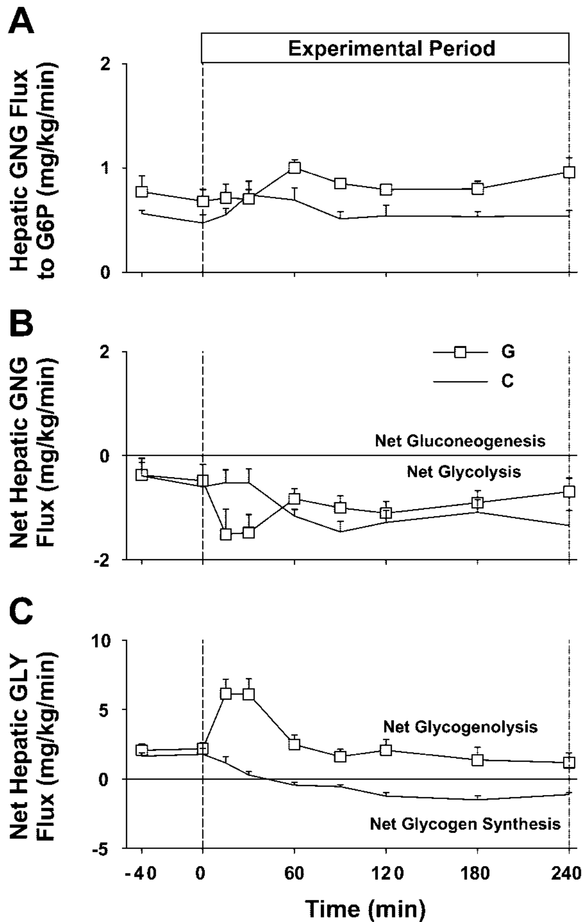

Fig. 4. Hepatic gluconeogenic (GNG) flux to glucose 6-phosphate (G-6-*P*; *A*), net hepatic GNG flux (*B*), and net hepatic glycogenolytic (GLY) flux (*C*) in control (−40 to 0 min) and experimental (0–240 min) periods in C and G 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated measures (significance accepted at $P < 0.05$); $n = 5$ for C and $n = 6$ for G. In C, GNG flux to G-6-*P* did not change, net hepatic GNG flux fell ($P < 0.05$), and net hepatic GLY flux fell ($P < 0.05$). In G, GNG flux to G-6-*P* did not change, net hepatic GNG flux fell ($P < 0.05$), and net hepatic GLY flux rose ($P < 0.05$) and then returned to basal levels. Among groups, for GNG flux to G-6-*P*, $P < 0.05$ for C vs. E; for net hepatic GNG flux, there were no significant differences; and for net hepatic GLY flux, $P < 0.05$ for C vs. G and G + E and for E vs. G + E.

glucose production and a marked effect on gluconeogenesis. In essence, we chose physiological levels of the two hormones that would produce large enough effects on glucose production to be significant alone but small enough to allow the detection of synergism if it occurred.

Our results confirm previous data that found additive effects of glucagon and epinephrine on tracer-determined glucose production (21, 62). However, in both previous studies the additive rise in the presence of both hormones was accompanied by an approximately twofold greater rise in peripheral insulin levels, in addition to an approximately twofold greater rise in the plasma glucose level, compared with the increments that occurred with either individual hormone (21). Therefore, it was possible that additive effects were observed in the previous studies only because hyperinsulinemia and hyperglycemia obscured the synergistic effects of the hormones. Unlike the previous studies, the present study controlled for insulin levels by use of a pancreatic clamp and glucose levels by use of a hyperglycemic clamp. Additionally, the present study separated glucose production into its gluconeogenic and glycogenolytic components. Despite the improved design, however, the conclusions remained the same.

Hepatic GNG flux to G-6-*P* changed as expected for the control group and for the individual-hormone treatment groups. Changes in net hepatic GNG flux closely resembled changes in GNG flux to G-6-*P*, even though absolute flux rates were lower. Glucagon treatment did not significantly increase either parameter, whereas epinephrine treatment increased both markedly. Com-

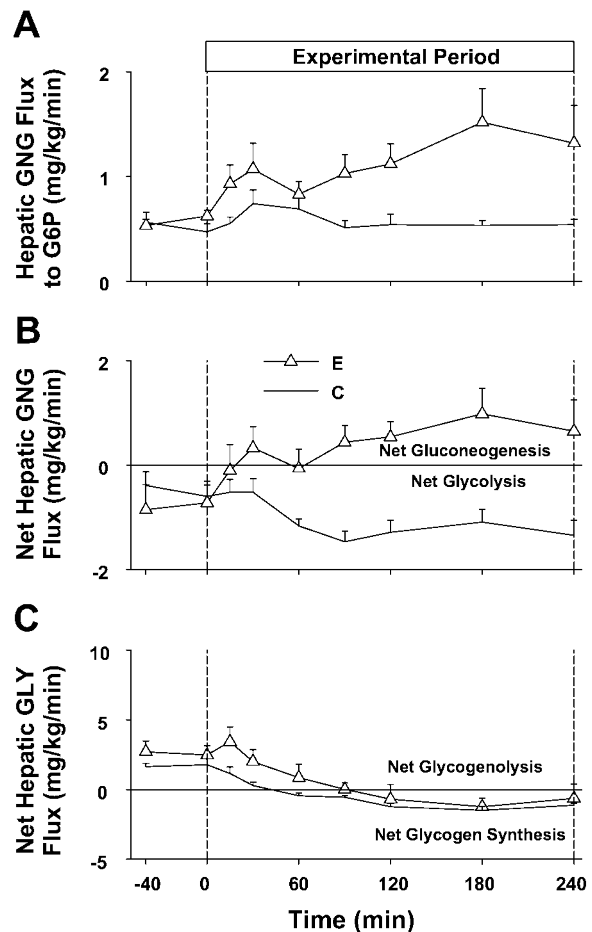

Fig. 5. Hepatic GNG flux to G-6-*P* (*A*), net hepatic GNG flux (*B*), and net hepatic GLY flux (*C*) in control (−40 to 0 min) and experimental (0–240 min) periods in C and E 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated measures (significance accepted at $P < 0.05$); $n = 5$ for C and $n = 6$ for E. In C, GNG flux to G-6-*P* did not change, net hepatic GNG flux fell ($P < 0.05$), and net hepatic GLY flux fell ($P < 0.05$). In E, GNG flux to G-6-*P* and net hepatic GNG flux rose ($P < 0.05$), and net hepatic GLY flux fell ($P < 0.05$). Among groups, for GNG flux to G-6-*P*, $P < 0.05$ for C vs. E; for net hepatic GNG flux, there were no significant differences; and for net hepatic GLY flux, $P < 0.05$ for C vs. G and G + E and for E vs. G + E.

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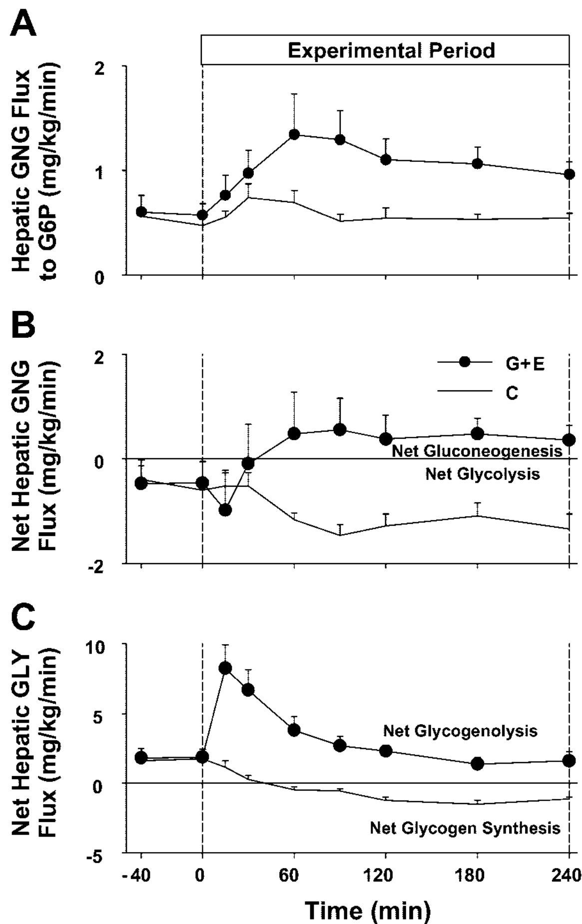

Fig. 6. Hepatic GNG flux (*A*), net hepatic GNG flux to G-6-*P* (*B*), and net hepatic GLY (*C*) in control (−40 to 0 min) and experimental (0–240 min) periods in C and G + E 18-h-fasted conscious dogs. Data are expressed as means ± SE. Statistical comparisons were made by 2-way ANOVA with repeated measures (significance accepted at $P < 0.05$); $n = 5$ for C and $n = 6$ for G + E. In C, GNG flux to G-6-*P* did not change, net hepatic GNG flux fell ($P < 0.05$), and net hepatic GLY flux fell ($P < 0.05$). In G + E, GNG flux to G-6-*P* and net hepatic GNG flux rose ($P < 0.05$), and net hepatic GLY flux rose ($P < 0.05$) and then returned to basal levels. Among groups, for GNG flux to G-6-*P*, $P < 0.05$ for C vs. E; for net hepatic GNG flux, there were no significant differences; and for net hepatic GLY flux, $P < 0.05$ for C vs. G and G + E and for E vs. G + E.

bination of the two hormones did not result in a synergistic effect on gluconeogenesis. There are several possible reasons why synergism did not occur. First, both hyperglycemia and the increased glycogen breakdown that occurred when both hormones were coadministered would be expected to increase flux through the glycolytic pathway. This would, in turn, raise fructose-2,6-bisphosphate levels, making flux through G-6-*P* in the gluconeogenic direction less likely to occur (31, 50). Second, the gluconeogenic substrates lactate and alanine did not rise as high in the G + E group as in the E group (see below). The reduced availability of lactate and alanine may have limited the gluconeogenic response when the hormones were coadministered. Third, it is possible that there was a synergistic effect on GNG flux but it was too small to detect given the assumptions of the method used to estimate gluconeogenesis.

Net hepatic GLY flux also changed as expected in the control group and the individual hormone treatment groups. Net glycogen breakdown ceased in response to hyperglycemia; in fact, net glycogen synthesis occurred by the end of the study. The increase in glucagon resulted in a large increase in net glycogenolysis, whereas the increment in epinephrine did not increase net glycogenolysis significantly over the 4-h period. Net glycogenolysis increased to a similar extent during combined hormone infusion as during glucagon-alone administration. It is likely that the lack of synergism with regard to gluconeogenesis explains the lack of inhibition of glycogenolysis by the combination of the two hormones.

Glucose utilization increased in the control group due to hyperglycemia. However, glucose utilization tended to increase less in both the glucagon group [probably due to decreased glucose uptake by liver (7,

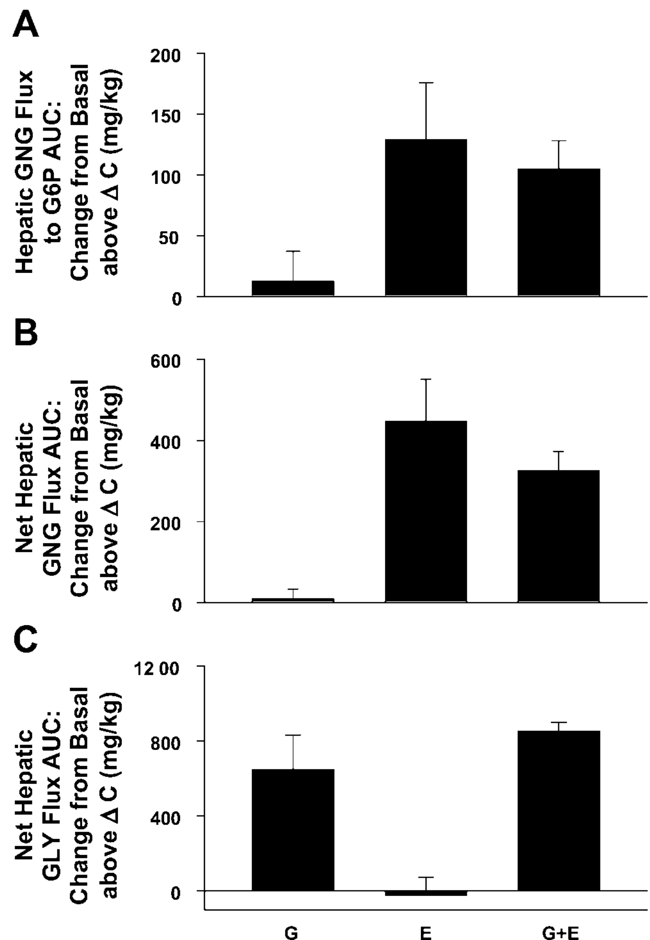

Fig. 7. AUCs of hepatic GNG flux to G-6-*P* (*A*), net hepatic GNG flux (*B*), and net hepatic GLY flux (*C*). All AUCs are shown as change from basal (ΔC), after subtraction of change from basal of C, over 4 h. Data are expressed as means ± SE. Statistical comparisons were made by 1-way ANOVA (significance accepted at $P < 0.05$); $n = 5$ for C, $n = 6$ each for G, E, and G + E. For GNG flux to G-6-*P*, $P < 0.05$ for G vs. E; for net GNG flux, $P < 0.05$ for G vs. E and G + E; and for net GLY flux, $P < 0.05$ for E vs. G and G + E.

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40, 51, 65)] and the epinephrine group [probably due to decreased glucose uptake by muscle (10)] than in the control group. When both hormones were combined, glucose utilization was significantly less than in the control (hyperglycemia alone) group. In fact, the increase in glucose utilization when both hormones were administered concurrently was not significant. This is important physiologically, because these hormones decrease glucose clearance individually by different mechanisms and thus together can increase glucose availability for the brain during times of stress.

Lactate levels rose in the hyperglycemic control group, as seen previously (65), likely due to increased glucose uptake by the liver and subsequent release of the carbon as lactate. Glucagon administration resulted in a small, quick rise in lactate production that waned with time, most likely the consequence of glucagon's rapid effect on glycogen breakdown, as reported previously (7, 8). This effect was short-lived, and after 1 h the glucagon group resembled the control group in both lactate levels and net hepatic balance. Lactate levels rose markedly with epinephrine treatment, as shown previously (6, 10, 14, 59), presumably due to increased lactate production from muscle glycogenolysis. Notably, lactate levels were significantly lower in the presence of both hormones than in the presence of epinephrine alone, even though both hormones stimulate lactate production by different organs. There are three possible explanations for this finding. The first relates to a known action of glucagon, which is to increase the efficiency of hepatic gluconeogenic precursor uptake (43, 67). In the combined-treatment group, the liver removed lactate at the same rate as in the epinephrine group, even though the arterial lactate level was much lower. Thus the liver was more efficient at removing lactate in the presence of both glucagon and epinephrine, probably because of stimulation of gluconeogenic enzyme activity by glucagon. This increased efficiency of uptake would likely allow steady state to be achieved earlier and thus result in a lower arterial lactate level. A second possible explanation for the lower lactate levels in the presence of both hormones is that lactate disappearance increased in response to glucagon at a site other than the liver. The third possible explanation is that glucagon decreased lactate appearance in the combined group. Because skeletal muscle has not been shown to possess glucagon receptors (4) and the kidney is not responsive to glucagon (25, 69), it seems unlikely that the effect on the lactate level was due to either of the latter possibilities.

Arterial alanine levels rose as expected in the control group due to hyperglycemia (65). Alanine levels remained unchanged in the presence of glucagon (67), the reason being that glucagon increases hepatic alanine fractional extraction by increasing alanine transport into the liver (36–38). As expected, alanine levels did not differ as a result of epinephrine treatment (10). However, when both hormones were administered together, the rise in alanine was less than with epinephrine alone. The possible explanations for this are the same as for lactate, with one additional possibility: the different lactate levels. Lactate administration increased alanine release from perfused rat skeletal muscle (58), and peripheral lactate infusion in the conscious dog increased the plasma alanine level (15). Thus alanine may have been lower in the combined group in part because lactate levels were lower.

Glycerol concentrations decreased in the control group, reflecting decreased lipolysis probably due to both hyperglycemia (16) and the infusion of somatostatin for an extended period of time (29). Glucagon is known to have little effect on lipolysis in vivo, and glucagon treatment had no demonstrable effect on glycerol levels in this study (2, 27). Epinephrine increased glycerol levels but only for a brief period, as expected (10, 16). Combined hormone treatment logically resembled epinephrine treatment, and glycerol levels increased and waned to similar values. For all groups, net hepatic glycerol uptake paralleled changes in arterial levels. In general, NEFA levels and net hepatic uptake tended to follow the same patterns as glycerol. Note that in both groups receiving epinephrine infusion, NEFA levels and uptake rates increased and waned, as expected. However, the elevations in both the level and net hepatic uptake in the combined hormone group were sustained for a longer period than in the epinephrine-alone group. This was perhaps due to the higher lactate level in the epinephrine group, as lactate has been shown to cause a fall in NEFA levels in vitro (3) and in vivo in dogs (15, 32, 44) and humans (1). Interestingly, NEFA increases gluconeogenesis in vivo (5, 9, 13, 52, 68, 73), and during the period (time 60–90 min) in which NEFA tended to be elevated in the combined group, there was a tendency for the GNG flux rate to be increased.

In summary, glucagon and epinephrine had additive effects on glucose production and perhaps glucose utilization. Furthermore, these hormones had additive effects on hepatic glycogenolysis. There was no synergism with regard to gluconeogenesis, probably due to the fact that glucagon increased the efficiency of hepatic gluconeogenesis without increasing the delivery of gluconeogenic precursors to the liver from muscle and adipose tissue. Regardless, it can be concluded that epinephrine did not modify glucagon's effect on either glycogenolysis or gluconeogenesis. When raised concurrently, glucagon and epinephrine do what neither can do alone, namely increase both components of hepatic glucose production. Under stress conditions, such changes in glucagon and epinephrine would undoubtedly be accompanied by changes in insulin, and it remains to be seen whether, in the presence of hyperinsulinemia, their interaction would be altered.

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REFERENCES

1. **Ahlborg G, Hagenfeldt L, and Wahren J.** Influence of lactate infusion on glucose and FFA metabolism in man. *Scand J Clin Lab Invest* 36: 193–201, 1976.
2. **Bertin E, Arner P, Bolinder J, and Hagstrom-Toft E.** Action of glucagon and glucagon-like peptide-1-(7–36) amide on lipolysis in human subcutaneous adipose tissue and skeletal muscle in vivo. *J Clin Endocrinol Metab* 86: 1229–1234, 2001.
3. **Bjorntorp P.** The effect of lactic acid on adipose tissue metabolism in vitro. *Acta Med Scand* 178: 253–255, 1965.
4. **Burcelin R, Katz EB, and Charron MJ.** Molecular and cellular aspects of the glucagon receptor: role in diabetes and metabolism. *Diabetes Metab* 22: 373–396, 1996.
5. **Chen X, Iqbal N, and Boden G.** The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. *J Clin Invest* 103: 365–372, 1999.
6. **Cherrington AD, Fuchs H, Stevenson RW, Williams PE, Alberti KG, and Steiner KE.** Effect of epinephrine on glycogenolysis and gluconeogenesis in conscious overnight-fasted dogs. *Am J Physiol Endocrinol Metab* 247: E137–E144, 1984.
7. **Cherrington AD, Williams PE, Shulman GI, and Lacy WW.** Differential time course of glucagon's effect on glycogenolysis and gluconeogenesis in the conscious dog. *Diabetes* 30: 180–187, 1981.
8. **Chhibber VL, Soriano C, and Tayek JA.** Effects of low-dose and high-dose glucagon on glucose production and gluconeogenesis in humans. *Metabolism* 49: 39–46, 2000.
9. **Chu CA, Sherck SM, Igawa K, Sindelar DK, Neal DW, Emshwiller M, and Cherrington AD.** Effects of free fatty acids on hepatic glycogenolysis and gluconeogenesis in conscious dogs. *Am J Physiol Endocrinol Metab* 282: E402–E411, 2002.
10. **Chu CA, Sindelar DK, Neal DW, Allen EJ, Donahue EP, and Cherrington AD.** Comparison of the direct and indirect effects of epinephrine on hepatic glucose production. *J Clin Invest* 99: 1044–1056, 1997.
11. **Chu CA, Sindelar DK, Neal DW, and Cherrington AD.** Direct effects of catecholamines on hepatic glucose production in conscious dog are due to glycogenolysis. *Am J Physiol Endocrinol Metab* 271: E127–E137, 1996.
12. **Chu CA, Sindelar DK, Neal DW, and Cherrington AD.** Portal adrenergic blockade does not inhibit the gluconeogenic effects of circulating catecholamines on the liver. *Metabolism* 46: 458–465, 1997.
13. **Clore JN, Glickman PS, Nestler JE, and Blackard WG.** In vivo evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. *Am J Physiol Endocrinol Metab* 261: E425–E429, 1991.
14. **Clutter WE, Bier DM, Shah SD, and Cryer PE.** Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. *J Clin Invest* 66: 94–101, 1980.
15. **Connolly CC, Stevenson RW, Neal DW, Wasserman DH, and Cherrington AD.** The effects of lactate loading on alanine and glucose metabolism in the conscious dog. *Metabolism* 42: 154–161, 1993.
16. **Coppack SW, Jensen MD, and Miles JM.** In vivo regulation of lipolysis in humans. *J Lipid Res* 35: 177–193, 1994.
17. **DeBodo RC, Steele F, Altszuler N, Dunn A, and Bishop JS.** On the hormonal regulation of carbohydrate metabolism: studies with 14 C glucose. *Recent Prog Horm Res* 19: 445–488, 1963.
18. **Diamond MP, Rollings RC, Steiner KE, Williams PE, Lacy WW, and Cherrington AD.** Effect of alanine concentration independent of changes in insulin and glucagon on alanine and glucose homeostasis in the conscious dog. *Metabolism* 37: 28–33, 1988.
19. **Dobbins RL, Davis SN, Neal D, Caumo A, Cobelli C, and Cherrington AD.** Rates of glucagon activation and deactivation of hepatic glucose production in conscious dogs. *Metabolism* 47: 135–142, 1998.
20. **Dobbins RL, Davis SN, Neal DW, Cobelli C, and Cherrington AD.** Pulsatility does not alter the response to a physiological increment in glucagon in the conscious dog. *Am J Physiol Endocrinol Metab* 266: E467–E478, 1994.
21. **Eigler N, Sacca L, and Sherwin RS.** Synergistic interactions of physiologic increments of glucagon, epinephrine, and cortisol in the dog: a model for stress-induced hyperglycemia. *J Clin Invest* 63: 114–123, 1979.
22. **Exton JH.** Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab Rev* 3: 163–183, 1987.
23. **Fosgerau K, Mittelman SD, Sunehag A, Dea MK, Lundgren K, and Bergman RN.** Lack of hepatic "interregulation" during inhibition of glycogenolysis in a canine model. *Am J Physiol Endocrinol Metab* 281: E375–E383, 2001.
24. **Gerich JE.** Control of glycaemia. *Baillieres Clin Endocrinol Metab* 7: 551–586, 1993.
25. **Gerich JE, Meyer C, and Stumvoll MW.** Hormonal control of renal and systemic glutamine metabolism. *J Nutr* 130: 995S–1001S, 2000.
26. **Goldstein RE, Rossetti L, Palmer BA, Liu R, Massillon D, Scott M, Neal D, Williams P, Peeler B, and Cherrington AD.** Effects of fasting and glucocorticoids on hepatic gluconeogenesis assessed using two independent methods in vivo. *Am J Physiol Endocrinol Metab* 283: E946–E957, 2002.
27. **Gravholt CH, Moller N, Jensen MD, Christiansen JS, and Schmitz O.** Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. *J Clin Endocrinol Metab* 86: 2085–2089, 2001.
28. **Hamilton KS, Gibbons FK, Bracy DP, Lacy DB, Cherrington AD, and Wasserman DH.** Effect of prior exercise on the partitioning of an intestinal glucose load between splanchnic bed and skeletal muscle. *J Clin Invest* 98: 125–135, 1996.
29. **Hendrick GK, Frizzell RT, and Cherrington AD.** Effect of somatostatin on nonesterified fatty acid levels modifies glucose homeostasis during fasting. *Am J Physiol Endocrinol Metab* 253: E443–E452, 1987.
30. **Hopgood MF, Clark MG, and Ballard FJ.** Protein degradation in hepatocyte monolayers. Effects of glucagon, adenosine 3′:5′-cyclic monophosphate and insulin. *Biochem J* 186: 71–79, 1980.
31. **Hue L.** Regulation of gluconeogenesis in the liver. In: *Handbook of Physiology: The Endocrine Pancreas and Regulation of Metabolism*. Bethesda, MD: Am. Physiol. Soc., 2001, vol. II, sect. 7, chapt. 20, p. 649–657.
32. **Issekutz B and Miller H.** Plasma free fatty acids during exercise and the effect of lactic acid. *Proc Soc Exp Biol Med* 110: 237–239, 1962.
33. **Jahoor F, Peters EJ, and Wolfe RR.** The relationship between gluconeogenic substrate supply and glucose production in humans. *Am J Physiol Endocrinol Metab* 258: E288–E296, 1990.
34. **Jenssen T, Nurjhan N, Consoli A, and Gerich JE.** Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic autoregulation without a change in plasma glucose concentration. *J Clin Invest* 86: 489–497, 1990.
35. **Jungermann K and Katz N.** Functional specialization of different hepatocyte populations. *Physiol Rev* 69: 708–764, 1989.
36. **Kilberg MS.** Amino acid transport in isolated rat hepatocytes. *J Membr Biol* 69: 1–12, 1982.
37. **Kilberg MS, Barber EF, and Handlogten ME.** Characteristics and hormonal regulation of amino acid transport system A in isolated rat hepatocytes. *Curr Top Cell Regul* 25: 133–163, 1985.
38. **Kilberg MS, Stevens BR, and Novak DA.** Recent advances in mammalian amino acid transport. *Annu Rev Nutr* 13: 137–165, 1993.
39. **Kraus-Friedmann N and Feng L.** The role of intracellular Ca2+ in the regulation of gluconeogenesis. *Metabolism* 45: 389–403, 1996.
40. **Liljenquist JE, Bomboy JD, Lewis SB, Sinclair-Smith BC, Felts PW, Lacy WW, Crofford OB, and Liddle GW.** Effect of glucagon on net splanchnic cyclic AMP production in normal and diabetic men. *J Clin Invest* 53: 198–204, 1974.

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41. **Magnusson I, Rothman DL, Gerard DP, Katz LD, and Shulman GI.** Contribution of hepatic glycogenolysis to glucose production in humans in response to a physiological increase in plasma glucagon concentration. *Diabetes* 44: 185–189, 1995.
42. **Mari A.** Estimation of the rate of appearance in the nonsteady state with a two-compartment model. *Am J Physiol Endocrinol Metab* 263: E400–E415, 1992.
43. **McGuinness OP, Burgin K, Moran C, Bracy D, and Cherrington AD.** Role of glucagon in the metabolic response to stress hormone infusion in the conscious dog. *Am J Physiol Endocrinol Metab* 266: E438–E447, 1994.
44. **Miller H, Issekutz B, and Rodahl K.** Effect of exercise on the metabolism of fatty acids in the dog. *Am J Physiol* 205: 167–172, 1963.
45. **Mortimore GE, Poso AR, Kadowaki M, and Wert JJ Jr.** Multiphasic control of hepatic protein degradation by regulatory amino acids. General features and hormonal modulation. *J Biol Chem* 262: 16322–16327, 1987.
46. **Mortimore GE, Poso AR, and Lardeux BR.** Mechanism and regulation of protein degradation in liver. *Diabetes Metab Rev* 5: 49–70, 1989.
47. **Myers SR, Biggers DW, Neal DW, and Cherrington AD.** Intraportal glucose delivery enhances the effects of hepatic glucose load on net hepatic glucose uptake in vivo. *J Clin Invest* 88: 158–167, 1991.
48. **Parrilla R, Goodman MN, and Toews CJ.** Effect of glucagon: insulin ratios on hepatic metabolism. *Diabetes* 23: 725–731, 1974.
49. **Pilkis SJ, Claus TH, and el-Maghrabi MR.** The role of cyclic AMP in rapid and long-term regulation of gluconeogenesis and glycolysis. *Adv Second Messenger Phosphoprotein Res* 22: 175–191, 1988.
50. **Pilkis SJ and Granner DK.** Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54: 885–909, 1992.
51. **Pozefsky T, Tancredi RG, Moxley RT, Dupre J, and Tobin JD.** Metabolism of forearm tissues in man. Studies with glucagon. *Diabetes* 25: 128–135, 1976.
52. **Puhakainen I and Yki-Jarvinen H.** Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. *Diabetes* 42: 1694–1699, 1993.
53. **Radziuk J and Pye S.** Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. *Diabetes Metab Res Rev* 17: 250–272, 2001.
54. **Randle PJ.** Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 14: 263–283, 1998.
55. **Randle PJ, Kerbey AL, and Espinal J.** Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. *Diabetes Metab Rev* 4: 623–638, 1988.
56. **Rizza R, Haymond M, Cryer P, and Gerich J.** Differential effects of epinephrine on glucose production and disposal in man. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 237: E356–E362, 1979.
57. **Rosa F.** Ultrastructural changes produced by glucagon, cyclic 3′5′-AMP and epinephrine on perfused rat livers. *J Ultrastruct Res* 34: 205–213, 1971.
58. **Ruderman NB and Berger M.** The formation of glutamine and alanine in skeletal muscle. *J Biol Chem* 249: 5500–5506, 1974.
59. **Sacca L, Vigorito C, Cicala M, Corso G, and Sherwin RS.** Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. *Am J Physiol Endocrinol Metab* 245: E294–E302, 1983.
60. **Satake S, Moore MC, Igawa K, Converse M, Farmer B, Neal DW, and Cherrington AD.** Direct and indirect effects of insulin on glucose uptake and storage by the liver. *Diabetes* 51: 1663–1671, 2002.
61. **Schworer CM and Mortimore GE.** Glucagon-induced autophagy and proteolysis in rat liver: mediation by selective deprivation of intracellular amino acids. *Proc Natl Acad Sci USA* 76: 3169–3173, 1979.
62. **Shamoon H, Hendler R, and Sherwin RS.** Synergistic interactions among antiinsulin hormones in the pathogenesis of stress hyperglycemia in humans. *J Clin Endocrinol Metab* 52: 1235–1241, 1981.
63. **Sherck SM, Shiota M, Saccomando J, Cardin S, Allen EJ, Hastings JR, Neal DW, Williams PE, and Cherrington AD.** Pancreatic response to mild non-insulin-induced hypoglycemia does not involve extrinsic neural input. *Diabetes* 50: 2487–2496, 2001.
64. **Shiota M, Jackson PA, Bischoff H, McCaleb M, Scott M, Monohan M, Neal DW, and Cherrington AD.** Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs. *Am J Physiol Endocrinol Metab* 273: E868–E879, 1997.
65. **Shulman GI, Lacy WW, Liljenquist JE, Keller U, Williams PE, and Cherrington AD.** Effect of glucose, independent of changes in insulin and glucagon secretion, on alanine metabolism in the conscious dog. *J Clin Invest* 65: 496–505, 1980.
66. **Stevenson RW, Steiner KE, Connolly CC, Fuchs H, Alberti KG, Williams PE, and Cherrington AD.** Dose-related effects of epinephrine on glucose production in conscious dogs. *Am J Physiol Endocrinol Metab* 260: E363–E370, 1991.
67. **Stevenson RW, Steiner KE, Davis MA, Hendrick GK, Williams PE, Lacy WW, Brown L, Donahue P, Lacy DB, and Cherrington AD.** Similar dose responsiveness of hepatic glycogenolysis and gluconeogenesis to glucagon in vivo. *Diabetes* 36: 382–389, 1987.
68. **Stingl H, Krssak M, Krebs M, Bischof MG, Nowotny P, Furnsinn C, Shulman GI, Waldhausl W, and Roden M.** Lipid-dependent control of hepatic glycogen stores in healthy humans. *Diabetologia* 44: 48–54, 2001.
69. **Stumvoll M, Meyer C, Kreider M, Perriello G, and Gerich J.** Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. *Metabolism* 47: 1227–1232, 1998.
70. **Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, and Stein DT.** Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol Endocrinol Metab* 276: E977–E989, 1999.
71. **Wahren J, Felig P, Ahlborg G, and Jorfeldt L.** Glucose metabolism during leg exercise in man. *J Clin Invest* 50: 2715–2725, 1971.
72. **Wall JS, Steele R, DeBodo RC, and Altszuler N.** Effect of insulin in utilization and production of circulating glucose. *Am J Physiol* 189: 43–50, 1957.
73. **Williamson JR, Kreisberg RA, and Felts PW.** Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci USA* 56: 247–254, 1966.
74. **Wolfe RR, Jahoor F, and Shaw JH.** Effect of alanine infusion on glucose and urea production in man. *J Parenter Enteral Nutr* 11: 109–111, 1987.
75. **Woodside KH, Ward WF, and Mortimore GE.** Effects of glucagon on general protein degradation and synthesis in perfused rat liver. *J Biol Chem* 249: 5458–5463, 1974.

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