Role of epinephrine and norepinephrine in the metabolic response to stress hormone infusion in the conscious dog

Owen P. McGuinness, Vickie Shau, Eric M. Benson, Mike Lewis, Robert T. Snowden, James E. Greene, Doss W. Neal, and Alan D. Cherrington

Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee 37232–0615

McGuinness, Owen P., Vickie Shau, Eric M. Benson, Mike Lewis, Robert T. Snowden, James E. Greene, Doss W. Neal, and Alan D. Cherrington. Role of epinephrine and norepinephrine in the metabolic response to stress hormone infusion in the conscious dog. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E674–E681, 1997.—The role of epinephrine and norepinephrine in contributing to the alterations in hepatic glucose metabolism during a 70-h stress hormone infusion (SHI) was investigated in four groups of chronically catheterized (20-h-fasted) conscious dogs. SHI increased glucagon (~5-fold), epinephrine (~10-fold), norepinephrine (~10-fold), and cortisol (~6-fold) levels. Dogs received either all the hormones (SHI; n = 5), all the hormones except epinephrine (SHI–Epi; n = 6), all the hormones except norepinephrine (SHI–NE; n = 6), or all the hormones except cortisol (SHI–C; n = 6). In addition, six dogs received saline only (Sal). Glucose production (Ra) and gluconeogenesis were assessed after a 70-h hormone or saline infusion with the use of tracer ([3-3H]glucose and [U-14C]alanine) and arteriovenous difference techniques. SHI increased glucose levels (108 ± 2 vs. 189 ± 10 mg/dl) and Ra (2.6 ± 0.2 vs. 4.1 ± 0.3 mg·kg⁻¹·min⁻¹) compared with Sal. The absence of an increase in epinephrine markedly attenuated these changes (glucose and Ra were 140 ± 6 mg/dl and 2.7 ± 0.4 mg·kg⁻¹·min⁻¹, respectively). Only 25% of the blunted increase in Ra could be accounted for by an attenuation of the rise in net hepatic gluconeogenic precursor uptake (0.9 ± 0.1, 1.5 ± 0.1, and 1.1 ± 0.2 mg·kg⁻¹·min⁻¹ for Sal, SHI, and SHI–Epi, respectively). The absence of an increase in norepinephrine did not blunt the rise in arterial glucose levels, Ra, or net hepatic gluconeogenic precursor uptake (they rose to 195 ± 21 mg/dl, 3.7 ± 0.5 mg·kg⁻¹·min⁻¹, and 1.7 ± 0.2 mg·kg⁻¹·min⁻¹, respectively). In summary, during chronic SHI, the rise in epinephrine exerts potent stimulatory effects on glucose production principally by enhancing hepatic glycogenolysis, although the rise in circulating norepinephrine has minimal effects.

THE METABOLIC RESPONSE to stress is accompanied by marked increases in counterregulatory hormones. We recently reported that chronic stress hormone infusion (SHI; a combined infusion of glucagon, epinephrine, norepinephrine, and cortisol for 70 h) in the dog created marked hyperglycemia and accelerated glucose production (Ra) (23). The increase in Ra was due to a combined increase in glycogenolysis (30%) and gluconeogenesis (70%). The latter resulted from increases in net hepatic gluconeogenic precursor uptake and the efficiency of hepatic gluconeogenesis as well as renal gluconeogenesis.

The role glucagon and cortisol play in this response has been addressed. During SHI, glucagon facilitates the gluconeogenic pathway by augmenting gluconeogenic precursor entry into the liver as well as by enhancing the efficiency of gluconeogenesis (23). In contrast, cortisol increases the supply of gluconeogenic precursors reaching the liver and augments hepatic glycogen stores despite concomitant elevations in the other counterregulatory hormones (14). To date, however, the role that elevated circulating catecholamine levels play in driving the metabolic response to SHI has not been examined.

Epinephrine is known to play an important role in augmenting hepatic glucose production during acute stresses (e.g., hypoglycemia and endotoxemia) (7, 15). The epinephrine-induced increase in glucose production is due both to an increase in hepatic glycogenolysis and gluconeogenesis. The latter is mainly driven by an increase in lactate delivery from peripheral tissues (30). Interestingly, although glucagon cannot effectively antagonize insulin suppression of glucose production (29), epinephrine is effective (31). As insulin levels increase in response to SHI, the potential for epinephrine to play a central role in the metabolic response to chronic stress may be enhanced compared with other counterregulatory hormones.

Circulating norepinephrine levels can also increase markedly in response to stress (8). H epatic glycogenolysis is less responsive to acute increases in circulating norepinephrine than epinephrine (8). However, hepatic gluconeogenesis is responsive to high levels of norepinephrine, such as can be seen at the adrenergic nerve terminal (~3,000 pg/ml). The enhancement in gluconeogenesis is due to combined increases in gluconeogenic precursor (lactate, glycerol) release by peripheral tissues, net hepatic fractional extraction of lactate by the liver, and net hepatic gluconeogenic efficiency.

The chronic effects of catecholamines on hepatic glucose metabolism have not been well studied, especially in settings in which other counterregulatory hormones are also elevated. Chronic β-adrenergic stimulation does not increase glucose production in humans (26). Yet during infection, in which multiple stress hormones are elevated, β-adrenergic blockade (propranolol) attenuates the stress-induced rise in Ra (18). The aim of the present study was to examine the impact the individual circulating catecholamines (epinephrine and norepinephrine) have in bringing about the metabolic response to chronic SHI.

METHODS

Animal preparation. Experiments were carried out on 20-h-fasted conscious mongrel dogs (23 ± 2 kg) receiving a diet consisting of Kal-Kan meat (Vernon, CA) and Purina dog chow (St. Louis, MO) once daily. The composition of the diet...
was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber, based on dry weight. The dogs 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.

Two weeks before an experiment, a laparotomy was performed with the animals under general anesthesia (acepromazine 0.55 mg/kg, pentobarbital sodium 25 mg/kg). Silastic catheters (0.03-in. ID; Dow Corning, Midland, MI) were placed into the inferior vena cava for the chronic infusion of hydrocortisone, epinephrine, and norepinephrine and into a splenic vein for the chronic infusion of glucagon. Blood sampling catheters (0.04-in. ID) were inserted into the femoral artery, the portal vein, the right renal vein, and the left hepatic vein for blood sampling, as previously described (23). In addition, Doppler flow probes were placed around the portal vein and the hepatic artery, after the gastroepiploic vein was ligated, to divert all portal venous drainage through the portal vein flow probe. The sampling and infusion catheters and the Doppler leads were placed under the skin before closure of the incision. The dogs received penicillin G intramuscularly (106 U) immediately after wound closure to minimize the possibility of infection. All animals studied had 1) a good appetite (consuming the entire ration), 2) normal stools, 3) a hematocrit above 35%, and 4) a leukocyte count below 18,000/mm².

Experimental design. On day 0 (14 days after surgery), after a 20-h fast, the subcutaneous ends of the infusion catheters were freed from their subcutaneous pockets through small skin incisions made with animals under local anesthesia (2% lidocaine), and the dog was placed in a jacket (Alice King Chatham, Los Angeles, CA) containing two pockets into each of which was placed a portable infusion pump (Auto Syringe, Travenol Laboratories, Hocksett, NH). Hydrocortisone was dissolved in saline and was infused with one pump at a rate of 4 µg·kg⁻¹·min⁻¹ (240 µl/h) into the inferior vena cava. Epinephrine and norepinephrine were dissolved in saline containing ascorbic acid (0.7 mg/ml), and both were infused at a rate of 0.08 µg·kg⁻¹·min⁻¹ (240 µl/h) into the inferior vena cava using the other pump. Glucagon (5 ng·kg⁻¹·min⁻¹ ≈ 4 ml/h) was infused into the portal vein with the use of a portable INFU-MED 200 infusion pump (Medex Ambulatory Systems, Broomfield, CO). Four groups of dogs were studied. One group was infused with all of the hormones (SH1; n = 5). Six dogs received all of the hormones except epinephrine (SH1–Epi), and six dogs received all of the hormones except norepinephrine (SH1–NE). Six dogs were infused with saline only (Saline). All the solutions were prepared and filtered (0.2 µm) under sterile conditions before infusion, as previously described (23). Fresh solutions were prepared every 12 h on each of the 3 infusion days. On the 3rd day, after an overnight (20-h) fast, the sampling catheters and flow probe leads were freed from their subcutaneous pockets with animals under a local anesthetic (2% lidocaine), and basal metabolism was assessed.

Experimental protocol. On day 3 an Angiocath (18 gauge; Deseret Medicine, Sandy, UT) was inserted percutaneously into a cephalic vein. A primed (50 µCi) constant infusion of purified [3-3H]glucose (0.4 µCi/min) and infusions of [U-13C]alanine (0.4 µCi/min), p-aminophenipic acid (0.3 mg·kg⁻¹·min⁻¹), and indocyanine green (0.1 mg·m⁻²·min⁻¹) began, using the right cephalic vein, and continued throughout the entire experiment. The experiment consisted of two periods, an equilibration period (120 to 0 min) and a basal period (0 to 60 min). Femoral artery and portal, renal, and hepatic vein blood samples were taken every 15 min during the basal period. At the end of the basal period, the dog was euthanized and liver biopsies were rapidly (within 3–5 min after euthanasia) taken from each lobe of the liver and immediately frozen in liquid nitrogen for later analysis of liver glycogen content and tracer glucose incorporation into glycogen. In the SH1 group an additional study was performed after the basal period. Thus glycogen data were not obtained in that group. Instead glycogen data from a previously reported SH1 group were therefore used for comparison (22).

Tracer methods and calculations. The rates of total glucose production and utilization were calculated according to the method of Wall et al. (33) as simplified by DeBodo et al. (11). Net hepatic glucose output was calculated using the formula [H – (Fp × A + Fp × P)] × HBF, where H, A, and P are the blood glucose concentrations in the hepatic vein, femoral artery, and portal vein, respectively, and Fp and Fp represent the fractional contribution of the hepatic artery and portal vein, respectively, to total hepatic blood flow (HBF). Plasma glucose concentrations were converted to whole blood concentrations using a correction factor of 0.73, as previously reported (24).

The same equation was used to calculate net hepatic substrate (lactate, alanine, and glyceral) output. However, because the liver generally was a net consumer of these substrates (i.e., negative output), the data are presented as positive values and denoted net uptake. Net hepatic fractional substrate extraction was calculated using the following formula: [(Fh × A + Fp × P) – H]/(Fh × A + Fp × P).

Net renal glucose and substrate output was calculated using the following formula: (R – A) × RBF, where R and A are renal vein and femoral artery blood glucose (or substrate) concentrations and RBF is renal blood flow. Net intestinal glucose (or substrate) output was calculated as above using the following formula: (P – A) × PBF, where P and A are as defined above and PBF is portal vein blood flow. To be consistent with net hepatic substrate uptake, the data for the intestine and kidney are also presented as net uptake.

Organ (hepatic and renal) [3-3H]glucose uptake was calculated as the product of the organ blood flow (HBF and RBF) and ratio of the organ [3-3H]glucose balance to the average inflowing glucose specific activity (22). This assumes that the specific activity of glucose at the site of glucose utilization is equal to the inflowing glucose specific activity. Even if this assumption is incorrect, the error is insignificant, because the outflowing glucose specific activity is within 10% of the inflowing glucose specific activity. Total organ (hepatic and renal) glucose production is equal to the sum of net organ glucose output and organ glucose uptake.

Hepatic gluconeogenic efficiency was calculated as the ratio of the whole body [14C]glucose production rate to the total net hepatic [14C]-labeled gluconeogenic precursor uptake rate (alanine and lactate). When the liver was a net producer of [14C]lactate, as was seen in the saline-infused group, the release of [14C]lactate was set equal to zero, as previously described (23). Because the contribution of the kidney to whole body [14C]glucose production (i.e., renal gluconeogenesis from [14C]alanine) is negligible (22), whole body [14C]glucose production is assumed to equal to hepatic [14C]glucose production. The above equation yields a minimal estimate of gluconeogenesis because of the dilution of the precursors in the oxaloacetate pool of the hepatocyte and because the assessment of gluconeogenesis is from only two precursors. The methods employed nevertheless allow us to bracket the true rate at which circulating gluconeogenic precursors are converted to glucose by the liver. A maximal estimate can be obtained by assuming that all of the gluconeogenic precursors...
taken up by the liver are completely converted to glucose. A minimal estimate can be obtained by multiplying this maximal estimate by the tracer-determined gluconeogenic efficiency (which is itself a minimal estimate, as discussed elsewhere (22)).

Processing of blood samples. The method for collection and immediate processing of blood samples has been previously described (5). Radioactivity in plasma glucose was measured using established methods (5). Blood lactate, glycerol, and alanine were analyzed using the method of Lloyd et al. (20).

Plasma glucose was assayed immediately using a Beckman glucose analyzer. Plasma treated with 500 kallikrein inhibitor units of Trasylol (FBA Pharmaceuticals, NY) was assayed for immunoreactive glucagon using 30K antisera of Aguilar-Parada et al. (1) (coefficient of variation [CV] of 8%). Immunoreactive insulin (34) was assayed using a sephadex-bound antibody technique (Pharmacia Diagnostics, Piscataway, NJ; CV of 11%). Plasma cortisol was assayed with Clinical Assays Gamma Cost radioimmunoassay (RIA) kit (CV of 6%) (12). Plasma collected from blood samples that were immediately treated with ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid and glutathione was assayed for epinephrine and norepinephrine using high-performance liquid chromatography (HPLC) techniques (CV of 14%) (21), as modified by Davis et al. (10). Doppler-determined blood flow was obtained with the use of an ultrasonic, range-gated, pulsed Doppler flow meter designed by Hartley et al. and described by Hartley et al. (16) and Ishida et al. (17). Indocyanine green dye was measured spectrophotometrically (810 nm) to estimate total hepatic blood flow (19). In cases in which Doppler flow probes were not functional (1 of 5, 2 of 6, 3 of 6, and 3 of 6 in SHI, SHI–Epi, SHI–NE, and Saline, respectively), indocyanine green dye was used to assess total hepatic blood flow, and the fractional contribution of hepatic artery blood flow to total hepatic blood flow was assumed to equal the mean determined with Doppler flow probes in the respective group. p-Aminohippuric acid was measured as described by Brun (2) to estimate renal blood flow. The labeled and unlabeled concentrations of plasma alanine and lactate were determined using a short column ion exchange chromatographic system (6). Hepatic glycogen content was determined using an enzymatic method (4).

Materials. Glucagon was purchased from Eli Lilly (Indianapolis, IN). Epinephrine, norepinephrine, and p-aminohippuric acid were obtained from Sigma Chemical (St. Louis, MO). Hydrocortisone was purchased from Abbott Laboratories (North Chicago, IL). Glucagon 30K antisera was obtained from Upjohn Diagnostics (Kalamazoo, MN). [3-3H]glucose (HPLC purified) and [U-14C]alanine were purchased from NEN Research Products (Wilmington, DE).

Data analysis. The reported data represent means ± SE of the average steady-state values during the basal period on day 3. Data were analyzed using analysis of variance with post hoc analysis using Tukey's honestly significant difference multiple comparisons procedure (Systat, Cambridge, MA).

RESULTS

Hormone levels and glucose metabolism. SHI increased arterial plasma glucagon, epinephrine, norepinephrine, and cortisol (Table 1) relative to the levels evident in saline-infused dogs. When epinephrine was not included in the SHI (SHI–Epi), the plasma epinephrine level was not altered, but the plasma glucagon, norepinephrine, and cortisol levels increased as expected. When norepinephrine was omitted from the SHI (SHI–NE), the plasma norepinephrine level decreased slightly, and the plasma glucagon, epinephrine, and cortisol levels increased as expected (Table 1).

SHI increased arterial plasma glucose levels in SHI and SHI–NE compared with the saline-infused group (P < 0.05; Fig. 1). In the absence of an increase in epinephrine, the plasma glucose levels were increased in SHI and SHI–NE (57 and 43%, respectively; P < 0.05) relative to saline infusion, although they did not increase in SHI–Epi. A corresponding twofold increase in the arterial plasma insulin level was observed in all three hormone-infused groups (Table 1; P < 0.05). The rates of glucose appearance and disappearance were increased in SHI and SHI–NE (57 and 43%, respectively; P < 0.05) relative to saline infusion, although they did not increase in SHI–Epi (Fig. 1). Similar effects were evident when net hepatic glucose output data were examined (Table 2). SHI increased hepatic glucose production but failed to significantly increase net hepatic glucose output (power = 0.84) because of greater than predicted variance in this group. Glucose clearance was not altered significantly by SHI with or without the individual catecholamines.

Hepatic gluconeogenesis. Total hepatic blood flow was similar in SHI, SHI–Epi, and Saline (28 ± 3, 29 ± 4, and 31 ± 2 ml·kg⁻¹·min⁻¹, respectively) but was mildly elevated in SHI–NE (40 ± 4 ml·kg⁻¹·min⁻¹).

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<tr>
<th>Table 1. Effect of a 3-day infusion of saline, SHI, SHI–Epi, or SHI–NE on arterial plasma insulin, glucagon, epinephrine, norepinephrine, and cortisol in 20-h-fasted conscious dogs</th>
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<td>Glucagon, pg/ml</td>
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<td>Cortisol, µg/dl</td>
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<td>Insulin, μU/ml</td>
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| Data are means ± SE. Saline, experimental group receiving saline infusion; SHI, group receiving stress hormone infusion; SHI–Epi, group receiving all stress hormones except epinephrine; SHI–NE, group receiving all stress hormones except norepinephrine. *Significantly different from saline-infused group (P < 0.05). |

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<th>Table 2. Effect of a 3-day infusion of saline, SHI, SHI–Epi, or SHI–NE on net hepatic glucose output, hepatic glucose uptake, and hepatic glucose production in 20-h-fasted conscious dogs</th>
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<td>Net hepatic glucose output</td>
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<td>Hepatic glucose uptake</td>
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<td>Hepatic glucose production</td>
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| Data are means ± SE in mg·kg⁻¹·min⁻¹. *Significantly different from saline-infused group (P < 0.05). |
Arterial lactate levels averaged 649 ± 85 µM in the saline-infused group and were not significantly altered by SHI, SHI-Epi, or SHI-NE (Fig. 2). The liver was not a significant net consumer of lactate in the saline-infused group. However, despite unchanged arterial lactate levels, the liver was a net consumer of lactate after SHI, SHI-Epi, and SHI-NE (Fig. 2). The increase in net hepatic lactate uptake in SHI-Epi was 30% of the increase seen in SHI and SHI-NE. The changes in net hepatic lactate uptake were also reflected in increases in the net fractional extraction of lactate by the liver.

In all three hormone-infused groups the arterial alanine levels fell (P < 0.05) in parallel to rises in net fractional hepatic alanine extraction (P < 0.05; Fig. 2). Consequently, net hepatic alanine uptake remained unaltered after SHI compared with the saline-infused group.

Arterial glycerol levels, net fractional glycerol extraction by the liver, and net hepatic glycerol uptake were not altered by SHI, SHI-Epi, or SHI-NE compared with the saline-infused group (Fig. 3). However, arterial glycerol levels were lower in SHI-Epi and SHI-NE compared with SHI (P < 0.05). In addition, net hepatic glycerol uptake was lower in SHI-Epi than in SHI (P < 0.05). SHI and SHI-NE did not alter nonesterified fatty acid (NEFA) levels or net hepatic NEFA uptake. The absence of an increase in epinephrine caused NEFA levels and net hepatic NEFA uptake to decrease compared with SHI (P < 0.05).

Net hepatic gluconeogenic precursor uptake and gluconeogenic efficiency increased in SHI and SHI-NE compared with Saline (P < 0.05) (Table 3). In the absence of an increase in epinephrine, these increments were attenuated.

Hepatic glycogen contents were 34 ± 7, 59 ± 15, 75 ± 12, and 42 ± 6 mg/g liver in Saline, SHI, SHI-Epi, and SHI-NE, respectively. Hepatic glycogen content was increased in SHI and SHI-Epi, but not in SHI-NE. The glycogen data for SHI were taken from a previously reported group (22).

Intestinal and renal glucose metabolism. Net intestinal glucose uptake was not altered by SHI (0.5 ± 0.1, 0.5 ± 0.1, 0.4 ± 0.1, and 0.9 ± 0.3 mg·kg⁻¹·min⁻¹ for Saline, SHI, SHI-Epi, and SHI-NE, respectively). Likewise, the production of lactate and alanine by the intestine was not altered by SHI (1.7 ± 0.9, 2.3 ± 0.3, 2.4 ± 0.7, and 1.6 ± 0.7 µmol·kg⁻¹·min⁻¹ for lactate and 1.3 ± 0.2, 0.6 ± 0.1, 0.5 ± 0.1, and 0.7 ± 0.1 µmol·kg⁻¹·min⁻¹ for alanine, respectively). There was minimal net glycerol exchange across the intestine (data not presented).

Renal glucose metabolism could not be assessed in the Saline and SHI groups because catheters placed in the renal vein did not remain patent in most dogs. In SHI-Epi and SHI-NE, the kidney was not a net producer of glucose. Net renal glucose outputs in SHI-Epi and SHI-NE were −0.5 ± 0.2 and −1.2 ± 0.4 mg·kg⁻¹·min⁻¹. Renal glucose uptake was 1.2 ± 0.5 and 1.2 ± 0.2 mg·kg⁻¹·min⁻¹. Thus renal glucose production was 0.7 ± 0.3 and 0.0 ± 0.5 mg·kg⁻¹·min⁻¹ in the two groups, respectively. In our previous report (22), renal glucose production was increased by SHI (0.3 ± 0.3 vs. 0.9 ± 0.4 mg·kg⁻¹·min⁻¹; Saline vs. SHI).
The rates of uptake of lactate (2.7 ± 0.9 and 2.5 ± 0.6 µmol·kg⁻¹·min⁻¹ for SHI-Epi and SHI-NE, respectively) and glycerol (0.7 ± 0.1 and 0.5 ± 0.1 µmol·kg⁻¹·min⁻¹) and the rate of production of alanine (0.4 ± 0.1 and 0.6 ± 0.1 µmol·kg⁻¹·min⁻¹) by the kidney were not altered by the omission of epinephrine or norepinephrine from the SHI.

**DISCUSSION**

During chronic (70-h) SHI, epinephrine plays an important role in sustaining the observed increase in glucose metabolism, although circulating norepinephrine appears to play little, if any, role. The absence of an increase in epinephrine attenuated the SHI-induced rise in both hepatic gluconeogenesis and glycoegenolysis. In contrast, the absence of an increase in circulating norepinephrine had no effect on the SHI-induced changes in glucose metabolism.

The SHI-induced increase in net hepatic glucose output requires a concomitant increase in both epinephrine and glucagon. In the acute setting, these two hormones, when combined, can overcome the antagonistic effects of insulin (25). Whether this effect persists chronically is unknown. Surprisingly, the combined chronic infusions of cortisol, glucagon, and norepinephrine (SHI-Epi) were unable to sustain an increase in net hepatic glucose output. The net release of glucose
by the liver reflects a balance between hepatic glucose production and hepatic glucose uptake. Because hepatic glucose uptake was unaffected by the infusion of glucagon, cortisol, and norepinephrine, it can be concluded that these hormones alone were unable to enhance glucose production. We previously observed that, when glucagon was omitted from the SHI infusion, net hepatic glucose output also did not increase (22). In that case, however, the failure to increase net hepatic glucose output resulted from an increase in hepatic glucose uptake that equaled the rise in hepatic glucose production. Thus both glucagon and epinephrine must increase to sustain an increase in net hepatic glucose output over a prolonged period.

Although, acutely, epinephrine has more potent stimulatory effects on gluconeogenesis than glucagon (30, 32), its chronic stimulatory effects on this process appear to be less substantive than that of glucagon when multiple stress hormones are increased. In the acute setting, epinephrine's ability to mobilize gluconeogenic precursors and NEFA from peripheral tissues is the sole mechanism by which it enhances gluconeogenesis (13, 30). Glucagon, on the other hand, relies on its ability to enhance both the uptake of gluconeogenic precursors by the liver and their intrahepatic conversion to glucose (29). It is by this mechanism that glucagon chronically stimulates gluconeogenesis during SHI (22). Despite epinephrine's known potent stimulatory effects on lactate release from peripheral tissues in the acute setting, the absence of an increase in epinephrine (SHI–Epi) in the present study led to only a modest fall in its arterial level and a more marked fall in net hepatic lactate uptake. The SHI-induced rise in gluconeogenesis (both net hepatic gluconeogenic precursor uptake and gluconeogenic efficiency) was attenuated by only ~50% when epinephrine was absent from the infusion. This is in unmistakable contrast to what was seen when glucagon was omitted from the SHI, namely that neither net hepatic glucagon precursor uptake nor hepatic gluconeogenic efficiency increased (22). Thus, although epinephrine does contribute to the SHI-induced increase in gluconeogenesis, glucagon is the primary hormone that facilitates this process in the chronic multihormone setting.

In contrast to its modest effect on gluconeogenesis, epinephrine plays a persistent and substantial role in sustaining the SHI-induced rise in hepatic glycogenolysis. Hepatic glucose production is equal to the sum of glycogenolysis and gluconeogenesis. In SHI–Epi gluconeogenesis increased even though hepatic glucose production did not increase significantly. Thus, in SHI–Epi, hepatic glycogenolysis may have fallen despite marked increases in plasma glucagon and norepinephrine. We previously reported that, when glucagon was not infused, the stress hormone-induced increase in hepatic glycogen mobilization persisted (22). These data suggest that glucagon does not contribute to the rise in hepatic glycogenolysis during SHI. The lack of an effect of glucagon on hepatic glycogenolysis during SHI is consistent with the potent inhibitory effects of insulin, which increases during SHI, on glucagon-stimulated hepatic glycogenolysis (29).

The primacy of epinephrine in driving hepatic glycogenolysis during SHI is also consistent with the observation that, in the acute setting, epinephrine can overcome the suppressive effect of insulin on hepatic glycogen breakdown (31). Thus the sustained rise in glycogenolysis seen during SHI is principally supported by the rise in epinephrine.

Hepatic glycogen stores increase in response to SHI. The omission of epinephrine from the SHI cocktail further increased liver glycogen stores (Δ16 mg/g). This increase is consistent with the attenuated rise in hepatic glycogenolysis seen when epinephrine was not infused. A similar increase was observed when glucagon was not infused during SHI (Δ29 mg/g) (14, 22). However, glucagon does this by limiting hepatic glucose uptake and subsequent glycogen synthesis rather than by increasing glycogen breakdown. Thus epinephrine and glucagon complement one another in attenuating the glycogen accretion mediated by excess cortisol.

Surprisingly, norepinephrine did not amplify the stress hormone-induced increase in hepatic glucose production. In the acute setting norepinephrine is considerably less potent than epinephrine in stimulating hepatic glycogenolysis (8, 30). However, norepinephrine can efficiently enhance hepatic gluconeogenesis by increasing gluconeogenic efficiency and by switching a liver that is releasing lactate to one that is consuming it (8). In the present study gluconeogenesis was already enhanced by the combined effects of glucagon and epinephrine. Thus the effects of norepinephrine on gluconeogenesis may be inconsequential. In fact, norepinephrine may not exert a sustained stimulatory effect on gluconeogenesis, because the combined infusions of epinephrine, norepinephrine, and cortisol were unable to enhance gluconeogenesis (22). Given that the hyperglycemia tended to be higher in SHI–NE than SHI, norepinephrine may exert a protective role in limiting hyperglycemia induced by SHI. Although this study addresses the impact on hepatic glucose metabolism of increases in circulating norepinephrine during SHI, the impact of norepinephrine released by the nerve terminal remains to be defined.

The SHI-induced increase in net fractional hepatic alanine extraction was not altered by the selective absence of a rise in either catecholamine. Although in an acute setting the infusion of norepinephrine can

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<th>Table 3. Effect of a 3-day infusion of saline, SHI, SHI–Epi, or SHI–NE upon maximal rates of gluconeogenesis, gluconeogenic efficiency, and minimal rate of gluconeogenesis in 20-h-fasted conscious dogs</th>
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<td>Maximum gluconeogenic rate (mg·kg⁻¹·min⁻¹)</td>
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<td>Minimum gluconeogenic rate (mg·kg⁻¹·min⁻¹)</td>
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Data are means ± SE. Rate values expressed as mg glucose·kg⁻¹·min⁻¹.*Significantly different from saline-infused group (P < 0.05).
increase net fractional hepatic alanine extraction (8), its role in enhancing net fractional hepatic alanine extraction during SHI appears to be minimal. We reported previously that the chronic absence of an increase in glucagon prevented the SHI-induced increase in net hepatic alanine fractional extraction (22). Thus the accompanying hyperglucagonemia seen during chronic stress is the primary determinant of the increase in hepatic amino acid transport.

Surprisingly, epinephrine had a greater effect on NEFA availability than did norepinephrine. Omission of norepinephrine had no effect on SHI-stimulated lipolysis or NEFA availability. In contrast, lipolysis and NEFA availability were decreased during SHI when the increase in epinephrine (SHI–Epi) was absent. Acute increases in norepinephrine have been shown to increase both glycerol and NEFA levels (9), although similar increases in epinephrine do not lead to a sustained rise in either glycerol or NEFA levels (28). This may be explained in part by the greater rise in the arterial glucose levels seen after epinephrine infusion. The greater hyperglycemia seen after epinephrine infusion may inhibit lipolysis and enhance reesterification, thereby lowering NEFA levels (3, 27). In the present study greater hyperglycemia cannot explain the attenuated response in SHI–Epi, because the arterial glucose levels were lower in SHI–Epi compared with SHI–NE. The mechanism for the differential chronic and acute effects of these circulating hormones on fat metabolism remains to be established.

The increase in norepinephrine may have contributed to the stress hormone-induced increase in renal glucose production. We have previously reported that SHI increased renal glucose production (0.3 to 0.9 mg·kg⁻¹·min⁻¹) and omission of glucagon prevented this increase (22). Note that in the present study, as well as in previous studies, the kidney remained a net consumer of glucose because of an increase in renal glucose uptake (0.5 to 1.8 mg·kg⁻¹·min⁻¹) (22). In the present study, in the absence of an increase in epinephrine, renal glucose production remained unaltered, although in SHI–NE renal glucose production was suppressed. These data, when combined with our previous report (22), suggest that both glucagon and norepinephrine contribute to the increase in renal glucose production seen during SHI.

Thus, of the two circulating catecholamines, epinephrine plays the greater role by augmenting hepatic glucose production. Although an acute increase in epinephrine augments hepatic glucose production by increasing gluconeogenesis, its chronic effects in a setting in which other stress hormones are elevated are quite different. It mediates its chronic effects principally by enhancing hepatic glycogenolysis.

Perspective. We have now completed a series of studies examining the chronic interaction of glucagon, cortisol, epinephrine, and norepinephrine in regulating carbohydrate metabolism in the conscious dog (14, 22, 23). These stress hormones work in concert to maintain hyperglycemia during chronic stress in part by increasing hepatic glucose production (both glycogenolysis and gluconeogenesis). Glucagon plays a central role by increasing the efficiency of hepatic gluconeogenesis and by facilitating gluconeogenic precursor entry into the liver. It also limits hepatic glucose uptake, thus allowing increases in gluconeogenesis to be manifest as a net increase in glucose release by the liver. Epinephrine also plays a central role by enhancing hepatic glycogenolysis. Despite its very potent acute effects on gluconeogenic precursor supply, these actions play a relatively minor role chronically. Cortisol augments hepatic glycogen stores despite marked increases in other counter-regulatory hormones. In addition, it maintains the gluconeogenic precursor supply, thus supporting the glucagon-and, to a lesser extent, epinephrine-mediated increase in gluconeogenesis. Interestingly, circulating norepinephrine does not play a major role in augmenting hepatic glucose metabolism during chronic stress. Rather, these hormones complement one another to allow an efficient stimulation of hepatic metabolism. These studies cannot address the relative importance of a given stress hormone in a particular stress, because the impact of an individual hormone will depend on the specific stress and the accompanying endocrine response.

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