Regulation of Ketogenesis by Epinephrine and Norepinephrine in the Overnight-fasted, Conscious Dog

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SUMMARY

The effects on ketogenesis and lipolysis of a norepinephrine (0.04 μg/kg-min), epinephrine (0.04 μg/kg-min), or saline infusion were examined in the overnight-fasted, conscious dog. Plasma insulin and glucagon levels were maintained constant by means of a somatostatin infusion (0.8 μg/kg-min) and intraportal replacement infusions of insulin and glucagon. In saline-infused dogs, plasma epinephrine (62 ± 8 pg/ml), norepinephrine (92 ± 29 pg/ml), blood glycerol (87 ± 10 μM), and plasma nonesterified fatty acid (NEFA) (0.82 ± 0.17 mM) levels did not change. Total blood ketone body levels tended to rise (62 ± 10 to 83 ± 11 μM) by 3 h as did total ketone body production (1.5 ± 0.4 to 2.2 ± 0.4 μmol/kg-min) over the same time interval. Norepinephrine infusion to produce plasma levels of 447 ± 86 pg/ml caused a sustained 50% rise in glycerol levels (66 ± 17 to 99 ± 15 μmol/L, $P < 0.05$) and 53% rise in nonesterified fatty acids (0.53 ± 0.07 to 0.81 ± 0.15 μmol/L, $P < 0.05$). Total ketone body levels rose by 43% (51 ± 8 to 73 ± 10 μmol/L) and ketone body production rose by a similar proportion (1.5 ± 0.2 to 2.2 ± 0.3 μmol/kg-min), changes that did not differ significantly from control animals. A similar increment in plasma epinephrine levels (75 ± 15 to 475 ± 60 pg/ml) caused glycerol levels to rise by 82% (105 ± 23 to 191 ± 26 μmol/L) in 30 min, but this rise was not sustained and the level fell to 146 ± 14 μmol/L by 120 min. Plasma nonesterified fatty acids rose from a basal value of 0.89 ± 0.19 mM to 1.25 ± 0.29 mM during the first 30 min, but fell to 0.60 ± 0.12 mM by 2 h. Ketone body levels remained unchanged (66 ± 10 μmol/L) and ketone body production declined (1.5 ± 0.3 to 1.0 ± 0.2 μmol/kg-min). These data indicate that (1) although the sustained increase in lipolysis caused by norepinephrine was greater than that caused by fasting alone, the ketogenic responses were not different, and (2) epinephrine has a transient lipolytic effect and an antiketogenic effect compared with controls. DIABETES 1985; 34:425–32.

Epinephrine and norepinephrine are both considered to be lipolytic and ketogenic hormones based on their ability to cause an increase in the circulating fatty acid level and the blood ketone concentration when they are infused in man[1-4] or dog.[5] Whether these changes are primarily due to direct effects of the catecholamine or are secondary to the changes that occur in the levels of circulating insulin or glucagon remains unclear. Infusion of either catecholamine in man or dog markedly affects the pancreatic secretion of insulin and glucagon,[1,6-10] usually increasing glucagon levels and causing a transient fall in insulin levels followed by a rise secondary to epinephrine-induced hyperglycemia. This sensitivity of insulin and glucagon secretion to circulating catecholamine levels has, thus, made it difficult to assess the direct lipolytic and ketogenic effects of epinephrine and norepinephrine in vivo. Lipolytic effects of epinephrine and norepinephrine have been documented in vitro[11] as well as in vivo.[1,12-14] It is, thus, likely that at least part of the ketogenic action of these hormones in vivo is secondary to catecholamine action on adipose tissue with increased NEFA substrate supply to the liver. In vitro work in the isolated liver cell[15-17] and the perfused liver[18] have also supported a direct stimulatory effect of catecholamines on hepatic ketogenesis, although in some cases[15,16] the magnitude of the effects have been small and the catecholamine concentrations required supraphysiologic. Recent work in the rat in vivo indeed showed an antiketogenic effect of epinephrine.[19] To clarify these discrepancies and to quantitate the effects of a physiologic catecholamine increment in vivo, we have examined the effects of a selective increase in either epinephrine or norepinephrine on lipolysis and ketogenesis in overnight-fasted,

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conscious dogs in which fixed basal levels of circulating insulin and glucagon were maintained by means of intraportal infusions of the two hormones and somatostatin (i.e., in the presence of a "pancreatic clamp").

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were carried out on overnight-fasted, conscious mongrel dogs (mean weight 22.0 kg, range 19.2–26.8 kg) of either sex that had been fed a diet of two parts Wayne Dog Chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) (25% crude protein, 9% crude fat, 5% crude fiber, 12% moisture, and 49% carbohydrate) and one part Kal-Kan meat diet (crude protein minimum 10%, crude fat minimum 4%, crude fiber maximum 1.5%, moisture maximum 78%) once daily for 2–3 wk before experimentation. Catheters were inserted into the splenic, portal, and hepatic veins and a femoral artery under general anesthesia at least 2 wk before the experiment as previously described.[20] The position of the catheter tips was verified at autopsy. On the day of the study, the free ends of the catheters were removed from their subcutaneous pockets under local anesthesia (2% lidocaine, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts), their contents were aspirated, and physiologic saline was infused through them at a slow rate until the experiment was begun. Angiocaths (18 gauge; Abbott Laboratories, North Chicago, Illinois) were inserted percutaneously into both cephalic veins and a saphenous vein. The arterial, portal, and hepatic catheters were used for blood sampling. Somatostatin was infused through the saphenous vein catheter, indocyanine green dye was given through one cephalic vein catheter, the selected catecholamine was infused through the other, and the pancreatic hormones, insulin and glucagon, were infused through the splenic vein catheter. After completion of the pre-experimental preparation, the animal was placed in a Pavlov harness where it stood calmly for 20–30 min before the start of the experiment.

On the day immediately preceding the experimental day, blood was withdrawn to determine the leukocyte count and hematocrit of the animal. Only dogs that had (1) a leukocyte count below 16,000 mm^3, (2) an hematocrit above 35%, (3) a good appetite (consuming at least two-thirds of the daily ration), and (4) normal stools were used.

Experimental design. Each experiment consisted of a 120-min equilibration and hormone adjustment period, a 40-min control period, and a 180-min test period. At t = −120 min, a constant infusion of indocyanine green dye (0.075 mg/m^2-min) was begun and continued throughout the study. At t = −90 min, a peripheral infusion of somatostatin (0.8 μg/kg-min) was started to inhibit endogenous insulin and glucagon secretion. Intraportal replacement infusions of insulin (0.25 mU/kg-min) and glucagon (0.65 ng/kg-min) were started simultaneously with initiation of the somatostatin infusion. The plasma glucose level was then monitored every 5 min and the rate of insulin infusion was adjusted until the plasma glucose level was stabilized at a euglycemic value. Once stabilization had been achieved, the hormone infusion rates were left unchanged and the 40-min control period was started. The mean basal rate of insulin infusion for all dogs was 0.21 ± 0.02 mU/kg-min. At t = 0 min, infusions of saline, epinephrine (0.04 μg/kg-min), or norepinephrine (0.04 μg/kg-min) were started via the cephalic vein catheter.

Processing of blood samples. Blood samples were drawn every 10 min during the control period and every 15 min thereafter. For the measurement of acetoacetate, 3-hydroxybutyrate, lactate, and glycerol, 1 ml of whole blood was added to 3 ml of chilled 4% (wt/vol) perchloric acid. These samples were then centrifuged for 10 minutes at 10,000 × *g*, and the supernatant was decanted and stored at −70°F until the assays were performed. Acetoacetate was assayed within 24 h.

Plasma nonesterified fatty acids were measured radioisotopically using a procedure developed by Ho et al.[21] 3-Hydroxybutyrate, lactate, and glycerol were measured enzymatically on perchloric acid extracts of whole blood using a Technicon autoanalyzer.[22] Blood acetoacetate levels were determined spectrophotometrically.[23] Plasma glucose concentration was determined with a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, California). Immunoreactive glucagon (IRG) was assayed using 30K antiserum[24] in plasma samples to which 500 U/ml of Trasylol had been added. Immunoreactive insulin (IRI) was measured by the Sephadex bound-antibody procedure.[25] Epinephrine and norepinephrine levels were determined in plasma using a single-isotope derivative method.[26] Plasma indocyanine green concentration was estimated by spectrophotometric measurement at 810 nm. The arterio-hepatic venous difference of this compound in conjunction with the hematocrit was used to calculate hepatic blood and plasma flow.[27]

Calculations. Hepatic substrate balance was determined by the arterio-venous difference technique. The proportion of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 28% and 72%, respectively.[28] The hepatic balance of a compound is calculated as the product of the difference between the hepatic vein concentration and the weighted inflowing concentration (H − [0.28A + 0.72P]) and hepatic blood or plasma flow (whichever is appropriate for the metabolite in question). Total ketone bodies refers to the sum of 3-hydroxybutyrate and acetoacetate.

Statistical comparisons were made using Student's *t*-tests.[29]

Materials. Insulin and glucagon were purchased from Eli Lilly and Company, Indianapolis, Indiana; Phadebas insulin radioimmunoassay kit was purchased from Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey; and Trasylol was obtained from RBA Pharmaceuticals, Inc., New York, New York. Glucagon 30K antiserum was bought from the University of Texas, Southwestern Medical School, and the standard and ^{125}I-labeled glucagon were obtained from Novo Research Institute, Copenhagen, Denmark. Insulin, glucagon, and somatostatin solutions were prepared immediately before use with physiologic saline and contained 0.3% bovine serum albumin. Catecholamine solutions were prepared immediately before use with physiologic saline that contained 0.3 mg/ml ascorbate. Cyclic somatostatin was obtained from Bachem, Inc., Torrance, California. The Cat-A-Kit used for the determination of epinephrine and norepinephrine was purchased from Upjohn, Inc., Kalamazoo,

Michigan. Indocyanine green dye was obtained from Hynson, Westcott and Dunning, Inc., Baltimore, Maryland.

RESULTS

Insulin, glucagon, epinephrine, and norepinephrine levels before and after catecholamine infusion. Figure 1 shows that insulin and glucagon levels remained unchanged when either saline (8 ± 1 μU/ml and 90 ± 12 pg/ml, respectively), epinephrine (13 ± 1 μU/ml and 141 ± 28 pg/ml, respectively), or norepinephrine (10 ± 1 μU/ml and 72 ± 6 pg/ml, respectively) were infused. Differences between infusates were not significant. Figure 2 shows that the epinephrine level rose 6–7-fold (75 ± 15 to 475 ± 60 pg/ml) in response to epinephrine infusion, while norepinephrine levels did not change (104 ± 15 pg/ml). Conversely, the norepinephrine level rose fourfold (111 ± 15 to 447 ± 86 pg/ml) in response to norepinephrine infusion, while epinephrine levels did not change (70 ± 11 pg/ml). Epinephrine and norepinephrine levels (62 ± 8 and 92 ± 29 pg/ml, respectively) did not change significantly during saline infusion.

Effect of catecholamines on plasma glucose and blood lactate levels. The changes in plasma glucose and blood lactate caused by selective increases in epinephrine or norepinephrine are shown in Table 1. Epinephrine caused the plasma glucose level to rise from 115 ± 10 to 160 ± 16 mg/dl ($P < 0.05$). Blood lactate also increased in response to epinephrine (0.62 ± 0.16 to 2.15 ± 3.3 mM, $P < 0.05$). By contrast, infusion of norepinephrine did not cause either plasma glucose (114 ± 4 mg/dl) or blood lactate (0.72 ± 0.1 mM) levels to change. Neither blood lactate (0.73 ± 0.14 mmol/L) nor plasma glucose (105 ± 5 mg/dl) changed significantly during saline infusion.

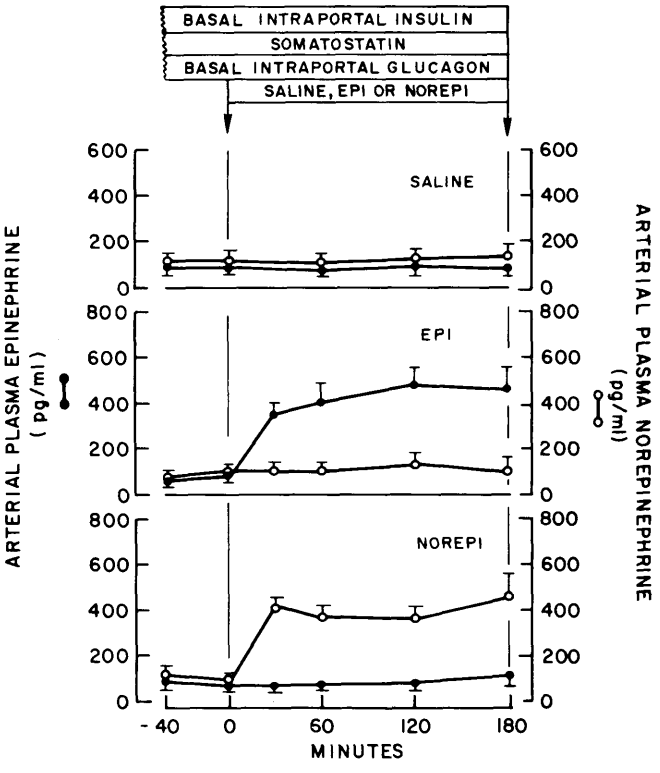

FIGURE 2. The effect of saline, epinephrine, or norepinephrine infusion (0.04 μg/kg-min) on arterial plasma epinephrine and norepinephrine levels in overnight-fasted, conscious dogs given somatostatin (0.8 μg/kg-min) and intraportal replacement infusions of insulin (233 μU/kg-min) and glucagon (0.65 ng/kg-min). Data are mean ± SEM.

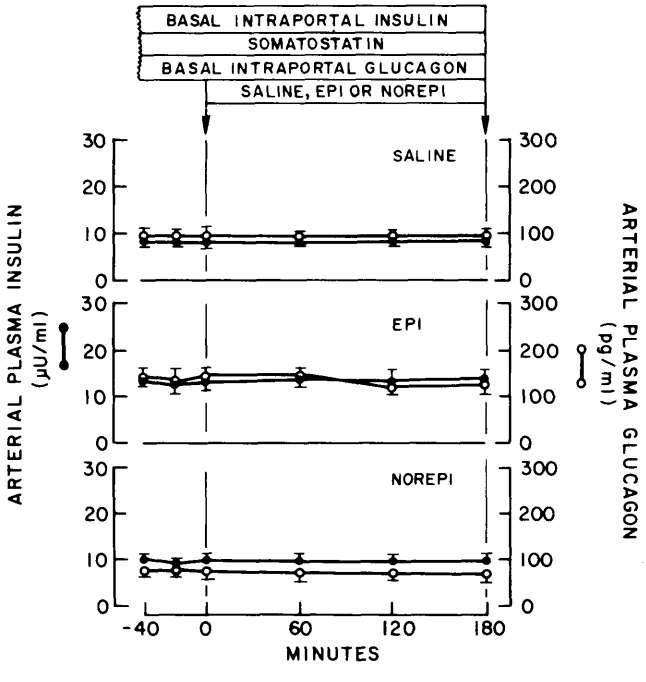

FIGURE 1. The effect of saline, epinephrine, or norepinephrine infusion (0.04 μg/kg-min) on arterial plasma insulin and glucagon levels in overnight-fasted, conscious dogs given somatostatin (0.8 μg/kg-min) and intraportal replacement infusions of insulin (208 μU/kg-min) and glucagon (0.65 ng/kg-min). Data are mean ± SEM.

Effect of catecholamines on arterial blood glycerol and plasma nonesterified fatty acid (NEFA) levels. The lipolytic effects of the catecholamines were assessed by monitoring both blood glycerol and plasma NEFA levels. Saline infusion had no significant effect on the blood glycerol level (87 ± 10 μmol/L, Figure 3), indicating that lipolysis did not change in these animals. Both catecholamines, however, caused a significant increase in lipolysis. Norepinephrine infusion was associated with a 50% rise in the blood glycerol level (66 ± 17 to 99 ± 15 μmol/L, $P < 0.05$), which was sustained through the entire 3-h period of catecholamine infusion (Figure 3). Epinephrine caused a more marked increase in the blood glycerol level with a peak after 1 h (105 ± 23 to 191 ± 26 μmol/L, $P < 0.05$); levels then fell to a plateau of 146 ± 14 μmol/L after 2 h.

Plasma nonesterified fatty acid levels (0.82 ± 0.17 mM) did not change significantly during infusion of saline, indicating stable lipolytic and re-esterification rates in the control group. Norepinephrine infusion caused a similar rise in NEFA levels to that found for glycerol (0.53 ± 0.07 to 0.81 ± 0.15 μM; + 53%; $P < 0.05$) within 30 min, levels remaining elevated thereafter. Epinephrine, on the other hand, caused a transient increase in NEFA levels from 0.89 ± 0.19 to 1.25 ± 0.29 mM, $P < 0.05$, during the first 30 min, but then a fall to subnormal levels by 120 min was observed.

TABLE 1
The responses of plasma glucose and whole blood lactate to an infusion of epinephrine or norepinephrine

	Minutes																
	−40	−30	−20	−10	0	15	30	45	60	75	90	105	120	135	150	165	180
Epinephrine																	
Plasma glucose (mg/dl)	117 ± 9	115 ± 10	115 ± 10	113 ± 10	113 ± 13	126 ± 14	134 ± 13	138 ± 14	144 ± 15	145 ± 15	152 ± 15	153 ± 16	158 ± 16	158 ± 16	159 ± 16	159 ± 16	160 ± 16
Blood lactate (mM)	0.65 ± 0.17		0.63 ± 0.16		0.59 ± 0.12		1.41 ± 0.29		1.96 ± 0.32		2.00 ± 0.34		1.93 ± 0.27		2.00 ± 0.28		2.15 ± 0.33
Norepinephrine																	
Plasma glucose (mg/dl)	114 ± 3	113 ± 4	115 ± 4	113 ± 4	113 ± 4	112 ± 4	112 ± 4	113 ± 5	114 ± 6		116 ± 6		117 ± 7		116 ± 8		115 ± 10
Blood lactate (mM)	0.72 ± 0.10		0.71 ± 0.10		0.76 ± 0.10		0.69 ± 0.08		0.70 ± 0.07		0.65 ± 0.05		0.63 ± 0.06		0.57 ± 0.05		0.59 ± 0.07

See text for details.

Effect of catecholamines on the arterial level and production of ketone bodies. The effects of a selective increase in either epinephrine or norepinephrine on total arterial blood ketone body levels and on hepatic ketone body production are shown in Figure 4. Both total ketone body levels and net hepatic production rose slowly over the course of the 3-h saline infusion (62 ± 10 to 83 ± 11 μmol/L and 1.5 ± 0.4 to 2.2 ± 0.4 μmol/kg-min, respectively). By contrast with the control group, the epinephrine infusion caused no change in total ketone body levels (66 ± 15 to 62 ± 12 μmol/L). Norepinephrine infusion, on the other hand, was associated with an elevation in blood total ketone body levels (43%; 58 ± 8 to 80 ± 10 μmol/L), but this was almost identical to that observed in the control group. In each case, the changes in arterial concentrations of total ketone bodies were a direct reflection of changes seen in hepatic ketone body production. During norepinephrine infusion, total ketone body production rose from a mean control period value of 1.5 ± 0.2 to 2.2 ± 0.3 μmol/kg-min during the last hour of the study. Epinephrine caused ketone body production to decline from a mean control period value of 1.5 ± 0.3 μmol/kg-min to an average of 1.0 ± 0.2 μmol/kg-min during the last hour, a value significantly below that observed in the control studies.

DISCUSSION

These studies were designed to determine the effects of a physiologic increment in the circulating levels of either epinephrine or norepinephrine on ketogenesis and lipolysis in vivo in the absence of changes in insulin and glucagon. An epinephrine infusion rate was chosen that caused the blood levels of the hormone to increase significantly, but remain within physiologic limits.[30,31] To compare the relative ketogenic effects of norepinephrine and epinephrine, the two hormones were infused at the same rate.

Changes in circulating levels of both glycerol and nonesterified fatty acid levels were measured to assess lipolytic effects. Glycerol levels will reflect lipolysis more purely, since glycerol cannot be used for re-esterification while measurement of plasma nonesterified fatty acids will reflect the balance between re-esterification and lipolysis. In both cases, changes in utilization will also affect circulating levels, and this caveat must always be borne in mind. Norepinephrine caused a sustained elevation of both glycerol (50%) and non-esterified fatty acids (53%), so that by either measure the hormone appeared to cause a significant and sustained increase in lipolysis. Glycerol uptake by the liver was in fact increased (data not shown), confirming that this rise must have been due to increased lipolysis. Previous studies in both dogs[5] and man[1,14] have shown that norepinephrine can stimulate lipolysis in vivo. In man, an infusion of 0.04 μg/kg-min caused a rise in NEFA similar to that seen in the studies presented here; however, after 50 min, the NEFA levels began to fall, presumably due to the rise in insulin that occurred at this time.[1] However, when somatostatin was infused together with norepinephrine, a sustained increase in glycerol and NEFA was found.[32] An infusion of norepinephrine twice that used in the present studies caused a twofold rise in NEFA levels that was sustained for the 90 min of study.[14] In the dog, a dose of norepinephrine greater than 10-fold that given in the present study caused a threefold increase in

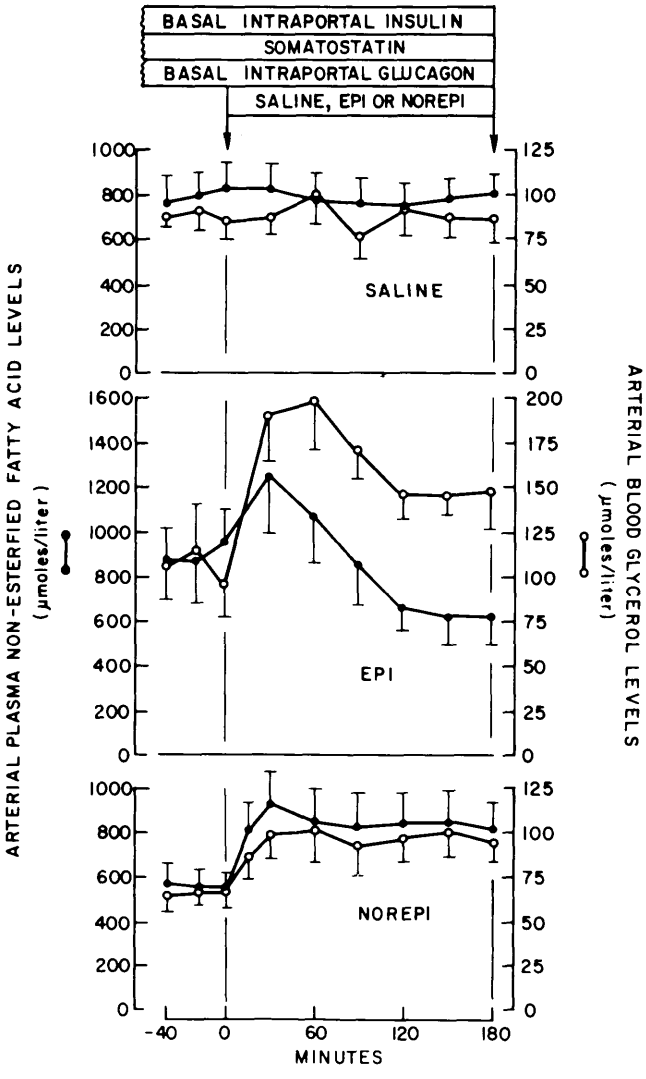

FIGURE 3. The effect of saline, epinephrine, or norepinephrine infusion (0.04 μg/kg-min) on arterial blood glycerol and plasma nonesterified fatty acid levels in overnight-fasted, conscious dogs given somatostatin (0.8 μg/kg-min) and intraportal replacement infusions of insulin and glucagon as in Figures 1 and 2. Data are mean ± SEM. The increments in glycerol over the mean control period value with epinephrine as compared with saline infusion are significantly different at 30, 60, and 90 min ($P < 0.05$) and with norepinephrine compared with saline at all points (30–180 min) ($P < 0.05$). The increments in NEFA over the mean control period value with epinephrine as compared with saline infusion are significantly different at 30 min ($P < 0.05$) and with norepinephrine compared with saline at 30 and 90 min ($P < 0.05$).

NEFA levels, but a time course was not shown and insulin was not controlled or measured so that no conclusions can be drawn relative to the ability of norepinephrine to sustain an increase in NEFA.

Epinephrine had a potent but transient lipolytic effect. The plasma glycerol level rose by 82% within 60 min and then fell to a value only 39% above the baseline. However, although its effect was biphasic, epinephrine continued to enhance lipolysis even up to 3 h. NEFA levels also showed a biphasic pattern such that the NEFA level was actually reduced below the control period value by 3 h. The greater proportionate fall in NEFA as compared with glycerol levels is probably a result of enhanced re-esterification. In other studies in diabetic man,[33] the baboon,[34] and the dog,[35] epinephrine induced an increase in plasma fatty acids; the studies in diabetic man and the dog were of short (20–60 min) duration, making it difficult to reach any conclusions about the ability of the hormone to cause a sustained increase in their levels. In one longer study in man with physiologic increases in epinephrine, a qualitatively similar biphasic effect was found although insulin and glucagon levels were not clamped.[36] In the rat, plasma NEFA levels were not altered by a high-dose epinephrine infusion plus somatostatin.[19]

The inhibition of lipolysis that was evident from the decline in glycerol and NEFA levels that occurred in the second and third hour of epinephrine infusion could be due to either a depletion of adipose tissue triglyceride, a highly unlikely event, a cellular "downregulation," or the increased presence of a circulating lipolytic inhibitor. Attenuation of the effect of epinephrine on adipose tissue may have occurred, since there is evidence of desensitization after exposure of adipose tissue to catecholamine.[37,38] However, if this had been the case, one might have expected that similar changes would have occurred with norepinephrine infusion, since the lipolytic action of both catecholamines is mediated by cAMP accumulation,[11] and there is evidence[37,38] that desensitization to both catecholamines can occur in adipose tissue.

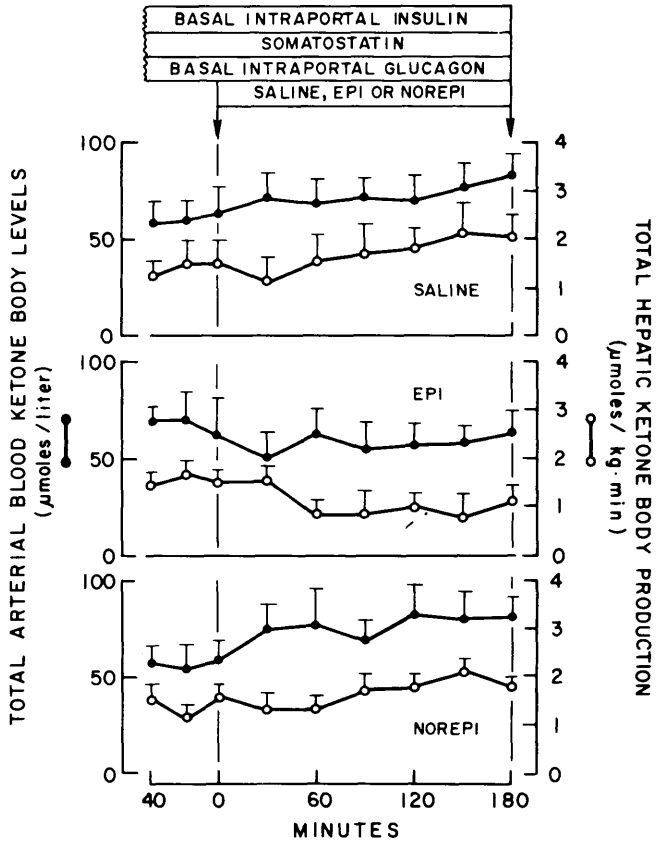

FIGURE 4. The effect of saline, epinephrine, or norepinephrine infusion (0.04 μg/kg-min) on total blood ketone body levels and hepatic ketone body production in overnight-fasted, conscious dogs given somatostatin and intraportal insulin and glucagon as in Figure 2. Data are mean ± SEM.

The most likely explanation for the changes observed in both glycerol and NEFA with epinephrine infusion relates to the increases in glucose and lactate observed with epinephrine but not with norepinephrine infusion. Evidence from both in vivo[39,40] and in vitro[41,42] studies indicate that increases in glucose and/or lactate concentrations similar to those seen in our studies can have potent antilipolytic effects. Indeed, lactate can inhibit catecholamine-induced lipolysis in vitro.[41,42] Plasma glucose level rose by approximately 40% (Table 1) with epinephrine infusion, while lactate levels increased by 347%. Such increases may overcome the stimulatory effect of epinephrine and result in a net inhibition of lipolysis. Infusion of lactate into the conscious, pancreatectomized dog[39] has been shown to cause a decline in lipolysis, while hyperglycemia has been shown to be antilipolytic in the conscious dog,[40] although in those studies lactate rose as well. However, in vitro studies[43] have shown glucose to be antilipolytic in isolated adipocytes. The transient rise and subsequent decline in NEFA and glycerol levels seen with epinephrine is, thus, probably due to lipolytic inhibition by lactate and/or glucose. However, the data also indicate that epinephrine may cause increased re-esterification of fatty acids, since lipolysis in the third hour was still elevated as indicated by the elevated glycerol levels, but NEFA levels at this time had fallen below control period values. These changes could also be due to enhanced utilization of NEFA other than for re-esterification or to a mass action effect of glucose in the adipocyte. In further support of an inhibitory role for glucose or lactate is the sustained effect of norepinephrine infusion on lipolysis with no changes in glucose or lactate levels.

To assess the changes in ketone body levels and production caused by the catecholamines, the results must be compared with those found in saline-infused control animals. Although saline-infused animals showed no increase in fatty acid levels, a slight rise in hepatic ketogenesis and total ketone body levels was observed as the study progressed. Such a change could not be related to changes in the insulin and glucagon levels, since they remained constant. It is more likely to be related to some intrinsic change in the liver that occurs on transition from the fed to fasted state. Recent evidence[44] has shown that the rate of gluconeogenesis can increase over the same period even when hormonal changes are prevented, indicating that nonhormonal factors may also alter hepatic carbohydrate metabolism during the progression of the fast.

Epinephrine infused at 0.04 μg/kg-min had an inhibitory effect on hepatic ketone production when compared with the rise in ketone body production seen in saline-infused animals. This has also been found by us in the rat[19] and may be due to several factors. There is some evidence in the perfused liver[45] for an inhibitory effect of epinephrine on NEFA uptake. This could account for a decrease in hepatic ketone production even in the presence of direct stimulatory effects of epinephrine on hepatic ketogenesis. Alternatively, epinephrine may have a direct stimulatory effect on ketogenesis in vivo at this dose, but it may only have been apparent after 30–60 min by which time NEFA levels had fallen below control period levels and would, thus, be reflected in a decreased uptake of NEFA. This decreased hepatic uptake of NEFA could effectively mask a stimulatory effect of epinephrine. Finally, epinephrine could have a direct inhibitory effect on hepatic ketogenesis in vivo, although in vitro evidence in perfused liver[18] and isolated liver cells[15–17] showed a small stimulatory, rather than inhibitory, effect of catecholamines on ketogenesis. However, in vivo, the rise in lactate will be paralleled by an increase in alanine and intracellular oxaloacetate that will divert acetyl CoA away from ketogenesis and into the tricarboxylic acid cycle. Alternatively, the apparent antiketogenic effect of epinephrine may relate to the ability of increased levels of glucose and lactate to stimulate lipogenesis[46] and thereby increase intracellular malonyl CoA levels, a potent inhibitor of carnitine palmitoyl-CoA transferase I. Inhibition of fatty acid transport into the mitochondria would be expected to decrease β-oxidation, reducing the rate of acetyl CoA formation and, consequently, ketogenesis. In support of such an inhibitory effect of epinephrine independent of changes in fatty acid delivery to the liver in these studies are the changes that occur in the ratio of NEFA concentration to hepatic ketone body production, a crude indicator of the hepatic efficiency of conversion of fatty acids to ketone bodies. In saline-infused animals, this ratio increased by 44% (1.88 to 2.71) from the control period to the third hour of saline infusion, while epinephrine infusion was associated with no change in this ratio (1.76 versus 1.73) over the same interval. Norepinephrine infusion caused a rise in ketone body levels and their production proportional to the rise in NEFA levels, but this rise was no different from the increases observed in saline-infused controls. The inability of norepinephrine to further stimulate ketogenesis (above controls) in the presence of a sustained increase in NEFA levels is more difficult to explain. However, even if norepinephrine prevented the rise in efficiency of conversion observed with saline infusion, a rise in delivery of fatty acids to the liver caused by the lipolytic effects of the hormone would still cause ketone body levels and their rate of production to increase proportionally to the rise in NEFA levels as was observed in these studies.

These studies used the somatostatin-pancreatic clamp technique to fix insulin and glucagon at basal levels. This peptide has been widely used to inhibit endocrine pancreatic function in vivo in metabolic studies. The use of this peptide in these studies assumes that it does not alter the effects of catecholamines on ketone body and fat metabolism. The lack of effects of somatostatin on epinephrine stimulation of fatty acid release has been reported.[47] Low-dose (0.1 μg/kg-min) infusion of somatostatin in man[47] concomitant with epinephrine infusion (0.07 μg/kg-min), when compared with subjects given only epinephrine, did not alter the ability of epinephrine to cause a rise in plasma cAMP, plasma glucose, and plasma fatty acids. It should be pointed out, however, that this low dose of somatostatin did not completely inhibit changes in insulin secretion. In addition, somatostatin had no effects on basal ketogenesis and lipolysis in either the overnight or 7-day fasted dog.[48] These reports, coupled with the fact that somatostatin does not alter catecholamine-induced changes in carbohydrate metabolism,[48–51] indicate that it can be used in conjunction with intraportal infusion of insulin and glucagon as an effective means to fix the levels of these hormones in this type of metabolic study.

In summary, these studies have defined the lipolytic and ketogenic effects of epinephrine and norepinephrine in the

overnight-fasted, conscious dog. Both catecholamines have lipolytic effects independent of changes in insulin and glucagon, although in the case of epinephrine these changes may be modulated by the increased production of glucose and lactate that act as lipolytic inhibitors. In spite of the initially increased flow of nonesterified fatty acid to the liver, epinephrine did not stimulate ketone body production and, thus, did not cause their levels to rise. Norepinephrine, however, was associated with a sustained increase in substrate supply and ketone production by the liver. The increase in ketone production caused by norepinephrine is most likely attributable to the increase in NEFA delivery to the liver, but, since saline infusion also caused a small increase in ketone body production in the absence of changes in glycerol and NEFA levels, it is possible that the rise in FFA actually offset a slight inhibitory action of norepinephrine on hepatic ketogenesis. In conclusion, epinephrine and norepinephrine in the overnight-fasted dog have lipolytic effects but do not directly stimulate hepatic ketogenesis in comparison with saline controls and, in fact, may have inhibitory effects on the ketogenic ability of the liver.

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