Synergistic Interactions of Physiologic Increments of Glucagon, Epinephrine, and Cortisol in the Dog

A MODEL FOR STRESS-INDUCED HYPERGLYCEMIA

NEAL EIGLER, LUIGI SACCÀ, and ROBERT S. SHERWIN, *Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510*

ABSTRACT To evaluate the role of anti-insulin hormone actions and interactions in the pathogenesis of stress-induced hyperglycemia, the counterregulatory hormones, glucagon, epinephrine, and cortisol were infused alone as well as in double and triple combinations into normal conscious dogs in doses that were designed to simulate changes observed in severe stress. Infusion of glucagon, epinephrine, or cortisol alone produced only mild or insignificant elevations in plasma glucose concentration. In contrast, the rise in plasma glucose produced by combined infusion of any two counterregulatory hormones was 50–215% greater ($P < 0.005$–0.001) than the sum of the respective individual infusions. Furthermore, when all three hormones were infused simultaneously, the increment in plasma glucose concentration (144±2 mg/dl) was two- to fourfold greater than the sum of the responses to the individual hormone infusions or the sum of any combination of double plus single hormone infusion ($P < 0.001$).

Infusion of glucagon or epinephrine alone resulted in a transient rise in glucose production (as measured by [3-^{3}H]glucose). While glucagon infusion was accompanied by a rise in glucose clearance, with epinephrine there was a sustained, 20% fall in glucose clearance. When epinephrine was infused together with glucagon, the rise in glucose production was additive, albeit transient. However, the inhibitory effect of epinephrine on glucose clearance predominated, thereby accounting for the exaggerated glycemic response to combined infusion of glucagon and epinephrine. Although infusion of cortisol alone had no effect on glucose production, the addition of cortisol markedly accentuated hyperglycemia produced by glucagon and(or) epinephrine primarily by sustaining the increases in glucose production produced by these hormones. The combined hormonal infusions had no effect on β-hydroxybutyrate concentration.

It is concluded that (*a*) physiologic increments in glucagon, epinephrine, and cortisol interact synergistically in the normal dog so as to rapidly produce marked fasting hyperglycemia; (*b*) in this interaction, epinephrine enhances glucagon-stimulated glucose output and interferes with glucose uptake while cortisol sustains elevations in glucose production produced by epinephrine and glucagon; and (*c*) these data indicate that changes in glucose metabolism in circumstances in which several counterregulatory hormones are elevated (e.g., "stress hyperglycemia") are a consequence of synergistic interactions among these hormones.

INTRODUCTION

It is well recognized that hyperglycemia and glucose intolerance may occur under conditions of severe stress, such as major trauma, or acute infection in patients without prior histories of diabetes (1–3). It is also well established that such circumstances are accompanied by marked increases in plasma glucagon, epinephrine, and cortisol (1–7). The precise relationship between stress-induced increments in these hormones and stress-induced hyperglycemia has not, however, been established. In fact, when glucagon or epinephrine are infused individually in physiologic doses, or when even pharmacologic quantities of glucocorticoids are administered, only modest changes in blood glucose regulation are observed in normal subjects (8–10). Inasmuch as counterregulatory hormones increase simultaneously during stress, the study of these hormones individually may be inadequate in assessing their impact on the development of hyper-

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glycemia. The possible role of synergistic interactions among physiologic increments in glucagon, epinephrine, and cortisol in the pathogenesis of stress-induced changes in glucose regulation has not been previously examined. Furthermore, whether physiologic hypercortisolemia acutely alters the hepatic response to glucagon or epinephrine has not been determined.

The present study was consequently undertaken to examine changes in plasma glucose and glucose kinetics produced by physiologic infusions of glucagon, epinephrine, and cortisol given individually or in combination. In the evaluation of glucose kinetics, special attention was given to the influence of cortisol on glucose production and the action of epinephrine on glucose uptake. The data indicate that counterregulatory hormones interact synergistically to produce marked hyperglycemia in the normal dog. Cortisol acts by maintaining glucose overproduction induced by glucagon and epinephrine, while epinephrine prevents compensatory increases in glucose uptake despite hyperinsulinemia.

METHODS

Experimental procedures. 40 experiments were performed on 15 normal conscious male dogs (19–29 kg) which had been fed standard Purina Dog Chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum for at least 7 d before study. Each dog received one to five infusion studies. Multiple studies in the same animal were performed in random order and were separated by an interval of at least 10 d. Dogs were studied in the postabsorptive state after a 16- to 18-h overnight fast. On the morning of the study, a polyethylene catheter was inserted percutaneously into a saphenous vein and advanced into an iliac vein for blood sampling. A cephalic vein was similarly cannulated for infusion of [3-^{3}H]glucose and glucoregulatory hormones.

A priming dose of [3-^{3}H]glucose (New England Nuclear, Boston, Mass.) was administered rapidly (at −2 h) followed by a continuous tracer infusion (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 80 nCi/min. The priming dose was 120-fold greater than the continuous infusion rate/min. A 2-h equilibration period (from −2 to 0 h) was employed to insure that plasma specific activity of the [3-^{3}H]glucose had reached a stable plateau before measurement of changes in glucose kinetics. The coefficient of variation of glucose specific activity during the final 20–30 min of the equilibration period (3–4 samples) in all experiments was 0.5–4.8% (2.0±0.2%, mean±SE). In studies employing cortisol in combination with glucagon and(or) epinephrine, the cortisol infusion was initiated 2 h before the addition of the other counterregulatory hormones. In these experiments, cortisol and [3-^{3}H]-glucose infusions were initiated at −2 h to reduce the length of the study. This was possible because infusion of cortisol alone had no effect on plasma glucose concentration or glucose kinetics (Results).

Seven groups of studies were performed. In three of the groups, a single counterregulatory hormone was infused: glucagon at a rate of 3.5 ng/kg body wt·min for 3 h (group 1, n = 7); epinephrine at a rate of 0.05 μg/kg·min for 5 h (group 2, n = 7); and cortisol as a primed-continuous infusion for 7 h (group 3, n = 4). The priming dose was given over the initial 90-min period at twice the continuous infusion rate. The continuous infusion dose was 200 μg/kg·h. In the remaining four groups of experiments, two or three counterregulatory hormones were infused in combination at rates identical to those used in experiments with single hormones: glucagon plus epinephrine (group 4, n = 6); cortisol plus glucagon (group 5, n = 4); cortisol plus epinephrine (group 6, n = 4); and cortisol plus glucagon plus epinephrine (group 7, n = 5). Blood samples were drawn at 10-min intervals before initiation of the counterregulatory hormone infusion (control values represent the mean of at least three preinfusion determinations), and at 5- to 30-min intervals thereafter until completion of the infusion at 3–7 h. Three additional dogs received an infusion of normal saline for 7 h.

Materials. Crystalline beef and pork glucagon (Eli Lilly and Company, Indianapolis, Ind.), epinephrine (Adrenaline, Park, Davis & Company, Detroit, Mich.), and cortisol sodium succinate (Solu-Cortef, The Upjohn Company, Kalamazoo, Mich.) infusates were freshly prepared in sterile, pyrogen-free saline. The glucagon infusate also contained homologous dog plasma (1 ml of plasma/50 ml of infusate), to prevent adherence of the glucagon to glassware or tubing, and aprotinin (1,000 kallikrein inactivator U/ml) (Trasylol, Delbay Pharmaceuticals Inc., Div. Schering Corp., Bloomfield, N. J.), to prevent breakdown of glucagon by plasma proteases. Ascorbic acid (30 mg/100 ml) was added to the epinephrine infusate to protect against oxidation.

Analyses. Plasma glucose concentration was measured by the glucose oxidase method with a Beckman Glucose Analyzer (Beckman Instruments, Inc., Fullerton, Calif.). The methods used for the determination of plasma immunoreactive insulin, plasma immunoreactive glucagon (with Unger antibody 30K), and blood β-hydroxybutyrate have previously been described (11, 12). Plasma cortisol was measured flourometrically (13). For the assay of [3-^{3}H]glucose radioactivity, plasma samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and the supernate was evaporated to dryness at 70°C to remove tritiated water. The dry residue was dissolved in 1 ml of water and counted with 10 ml of Aquasol (New England Nuclear) in a liquid scintillation spectrometer (Searle Diagnostics Inc., G. D. Searle & Co., Des Plaines, Ill.). Recovery of radioactive glucose (as determined by adding known amounts of radioactivity of plasma samples) averaged 96±2%.

Calculations. Rates of endogenous glucose production and uptake were calculated in the steady state before hormone infusion by the isotope dilution equation, and during nonsteady state conditions by Steele's equations in their derivative form (14). The time curves for glucose concentration and specific activity were fitted with polynomial functions by the method of least squares. The use of polynomial functions for the measurement of glucose kinetics in nonsteady states with Steele's equations has previously been validated (15). Polynomial equations were used solely in the calculation of glucose inflow and outflow rates; i.e., the glucose concentration data presented in the text and figures are true data points rather than idealized values derived from polynomial functions. The value of 0.65 (pool fraction) was used to correct for noninstantaneous mixing within the entire glucose pool (16). The evaluation of the rates of glucose turnover based on the primed continuous infusion and the pool fraction techniques has been validated for both steady and nonsteady states (15, 17). Glucose clearance was calculated as the ratio of the rate of glucose uptake and plasma glucose concentration and normalized to body weight (18). Glucose clearance has been used as an index of the ability of tissues to remove glucose from plasma independent of plasma glucose concentration (19). All calculations were performed on a Hewlett-Packard 9830A computer (Hewlett-Packard Co., Palo Alto, Calif.).

Statistical analyses were performed with the unpaired

Student's t test (the paired t test was used only in comparing changes from base line). To determine whether the changes in plasma glucose produced by combined hormone infusion were significantly different than the sum of the changes in plasma glucose caused by the components (single or double hormone infusion) of the combined infusion, an analysis of variance and covariance with a test of multiple comparisons between means and linear combinations of means was performed. Because multiple (seven) comparisons were made, a probability level of $P < 0.007$ was considered significant (20). Because the small sample size used precludes determining the normality of the population distribution (21), results of all parametric tests were corroborated with nonparametric tests of significance (Wilcox Rank Sum Test). Data in the text are presented as the mean±SE.

RESULTS

Effect of Single Counterregulatory Hormone Infusion on Glucose Metabolism

Glucagon. Infusion of glucagon alone resulted in a stable five- to sixfold elevation in plasma glucagon concentration within 20 min (Fig. 1). Mean plasma glucagon concentration throughout the glucagon infusion period was 219±3 pg/ml. As expected (8), plasma glucose (96±2 mg/dl, preinfusion) increased rapidly by 15–20 mg/dl and then gradually returned toward base line by 3 h despite ongoing glucagon administration (Fig. 2). Glucose production rose by 65–70% ($P < 0.001$) within 10–40 min and then gradually returned toward base-line levels. At the conclusion of the study, glucose production was slightly, but not significantly, increased as compared with values observed during the preinfusion period (Fig. 2). Glucose uptake also rose by 60% during the glucagon infusion, although the rate of rise was less rapid than that observed for glucose output (not shown). Similarly, glucose clearance increased by 25% ($P < 0.01$) and was accompanied by a twofold rise ($P < 0.01$) in plasma insulin concentration (19±2 μU/ml, preinfusion) (Fig. 2).

Epinephrine. In Fig 2, the response to infusion of epinephrine (5 h) alone is shown. Plasma glucose (93 ±2 mg/dl, preinfusion) rose progressively over 3 h and

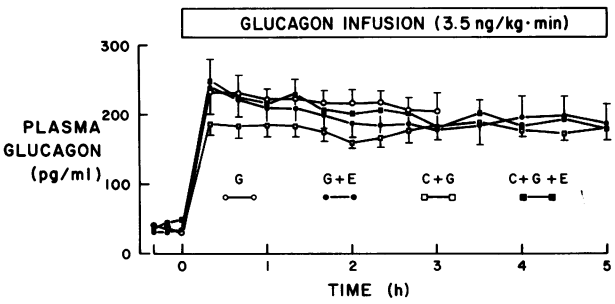

FIGURE 1 Plasma glucagon concentrations during the infusion of glucagon (G) alone or in combination with epinephrine (E) and(or) cortisol (C) in the normal, conscious dog.

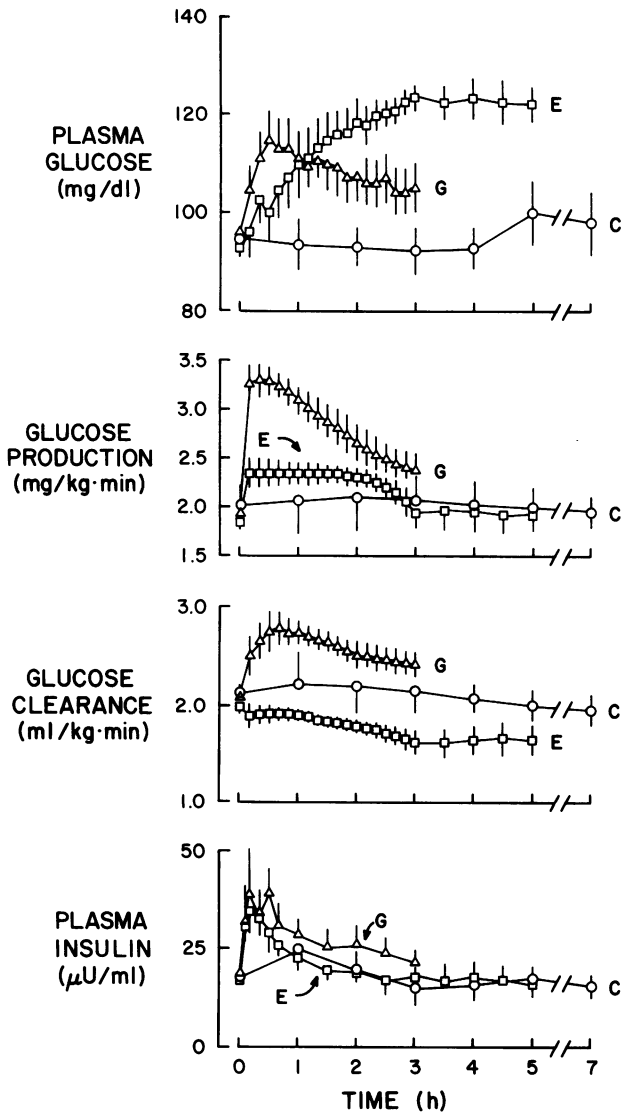

FIGURE 2 Effect of infusion of glucagon (G), epinephrine (E), and cortisol (C) individually on plasma glucose concentration, glucose production, glucose clearance, and plasma insulin concentration (mean±SE). The basal data (0 time) represent the mean of three observations at 10-min intervals.

then stabilized 28–30 mg/dl above base-line values. Epinephrine, like glucagon, produced a transient (though smaller) rise in glucose production ($P < 0.001$), which returned to base line within 3 h despite ongoing infusion of epinephrine. Glucose uptake, on the other hand, increased by only 10–15% ($P < 0.05$) and glucose clearance declined by 20% ($P < 0.02$), thereby accounting for the persistence of hyperglycemia (Fig. 2). Plasma insulin (18±1 μU/ml, preinfusion) rose by 75–100% ($P < 0.025$) within 5–10 min, although plasma glucose did not significantly increase until 20 min. However, after 60 min, plasma insulin levels

returned to preinfusion values despite the presence of hyperglycemia. Plasma glucagon (33±3 pg/ml in the basal state) was unchanged throughout the 5-h epinephrine infusion period.

Cortisol. Primed-continuous infusion of cortisol produced a sustained 200–250% ($P < 0.01$) increase in plasma cortisol concentration (8–10 μg/dl) after 2 h (Fig. 3). In contrast to glucagon and epinephrine, cortisol infusion had no significant effect on plasma glucose concentration (95±2 mg/dl, preinfusion), glucose production, uptake and clearance, or plasma insulin concentration throughout the entire 7-h study period (Fig. 2). In addition, plasma glucagon concentration (29±6 pg/ml in the basal state) was unchanged. Similarly, plasma glucose levels (97±3 mg/dl, preinfusion) were not significantly altered during saline administration (7 h). Furthermore, plasma glucose concentrations at the completion of the saline control (93±3 mg/dl) and cortisol (98±6 mg/dl) studies were not significantly different.

Effect of Combined Infusion of Counterregulatory Hormones on Glucose Metabolism

Glucagon plus epinephrine (G + E). Fig. 4 compares the effect of combined glucagon and epinephrine infusion with that observed during infusion of these hormones individually. Infusion of G + E produced a progressive increase in plasma glucose (92 ±1 mg/dl, preinfusion), reaching a plateau of 145–155 mg/dl in 80–100 min. The increment in plasma glucose during infusion of G + E was consistently greater than ($P < 0.05$–0.005) the algebraic sum of the responses to glucagon and epinephrine alone beyond 60 min. At the completion of the infusion, the rise in plasma glucose (58±3 mg/dl) was 50% greater than the sum of the responses to glucagon (10±2 mg/dl) and epinephrine

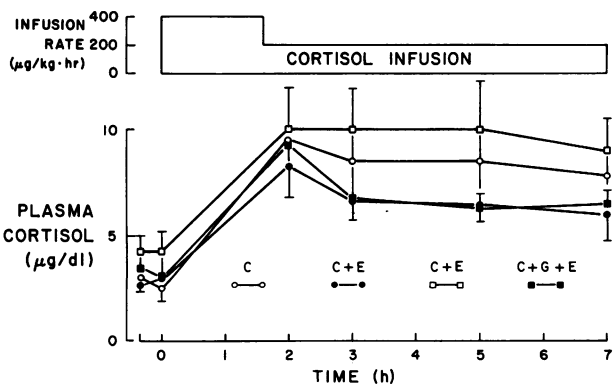

FIGURE 3 Changes in plasma cortisol during primed-continuous infusion of exogenous cortisol (C) alone or in combination with glucagon (G) and(or) epinephrine (E).

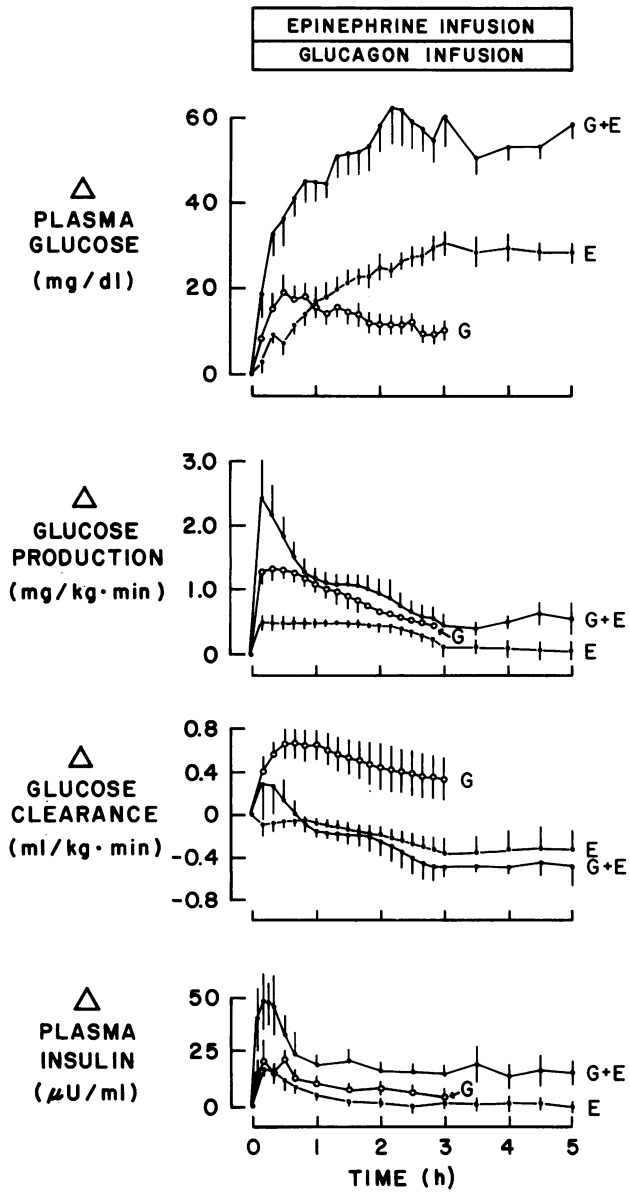

FIGURE 4 Changes in plasma glucose, glucose production, glucose clearance, and plasma insulin during combined infusion of glucagon plus epinephrine (G + E) as compared with infusion of glucagon (G) and epinephrine (E) individually.

(29±3 mg/dl) infused individually ($P < 0.001$). During G + E infusion, glucose production increased by 130% ($P < 0.02$) within 10 min and then returned toward preinfusion levels (Fig. 4). Beyond 3 h, glucose production was not significantly different from that observed during the preinfusion period. The rise in glucose clearance accompanying glucagon administration was prevented by the addition of epinephrine; glucose clearance declined to a similar extent to that observed with epinephrine alone (P = NS). Plasma insulin (17±1

μU/ml in the basal state) increased by 250–300% within 20 min, and remained 100% above preinfusion levels ($P < 0.02$) and 40–90% above those observed with glucagon or epinephrine given individually ($P < 0.05$). Plasma glucagon levels during the G + E infusion (180–240 pg/ml) were no different from those observed during infusion of glucagon alone (Fig. 1).

Cortisol plus glucagon (C + G). Plasma concentrations of cortisol and glucagon during C + G infusion were comparable to those observed during infusion of cortisol or glucagon alone (Figs. 1 and 3). The increment in plasma glucose produced by C + G consistently exceeded the sum of the changes observed during individual infusion of cortisol and glucagon after 100 min ($P < 0.05$) (Fig. 5). At the completion of the infusion, the rise in plasma glucose produced by C + G (41±8 mg/dl) was 215% greater than the sum of the responses to glucagon (10±2 mg/dl) and cortisol (3 + 4 mg/dl) alone ($P < 0.005$). Cortisol altered the effect of glucagon on glucose production primarily by sustaining elevations in glucose production produced by this hormone (Fig. 5). Glucose production remained 61% ($P < 0.001$) above preinfusion levels (2.0±0.1 mg/kg·min) after 5 h. While the initial (10–60 min) rise in glucose production which accompanied C + G infusion tended to be elevated as compared with that of glucagon alone, these changes were not significant ($P > 0.1$). In contrast, the addition of cortisol had no effect on glucagon-induced changes in glucose clearance or plasma insulin concentration (not shown).

Cortisol plus epinephrine (C + E). Infusion of C + E resulted in a rise in plasma cortisol to 8–10 μg/dl, comparable to that observed with cortisol alone. There was no change in plasma glucagon concentration (45±2 pg/ml, preinfusion). As shown in Fig. 5,

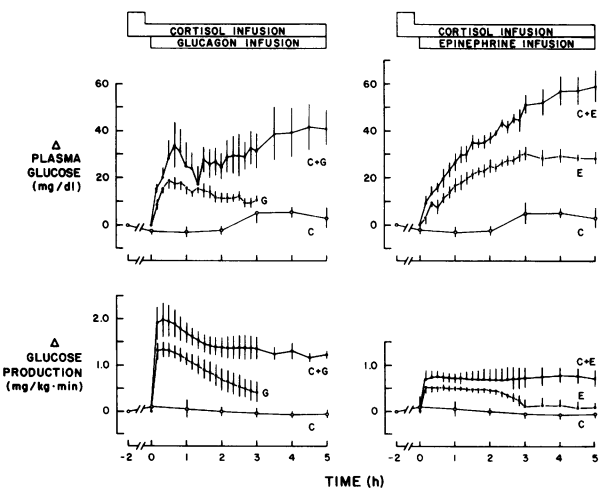

FIGURE 5 Effect of cortisol (C) on the response of plasma glucose and glucose production to glucagon (G) or epinephrine (E) infusion.

beyond 60 min, the rise in plasma glucose induced by C + E infusion was consistently greater than that observed with epinephrine alone ($P < 0.05$–0.001). Furthermore, after 5 h, the rise in plasma glucose caused by C + E (59±7 mg/dl) was twofold greater than the sum of the individual responses to epinephrine (29±3 mg/dl) and cortisol (3±4 mg/dl) ($P < 0.005$). As in the case of glucagon, cortisol exerted its effect by sustaining increases in glucose production induced by epinephrine. Glucose production remained 35–40% above preinfusion levels (1.9±0.1 mg/kg·min) throughout the 5-h study period and was significantly greater than that produced by epinephrine alone after 3 h ($P < 0.05$) (Fig. 5). In contrast, epinephrine-induced changes in glucose clearance and plasma insulin levels were not altered by the addition of cortisol (not shown).

Cortisol plus glucagon plus epinephrine (C + G + E). C + G + E infusion resulted in plasma concentrations of cortisol (7–9 μg/dl) and glucagon (180–250 pg/ml) which were not significantly different from those observed during infusion of cortisol or glucagon alone (Figs. 1 and 3). Fig. 6 compares the effect of combined C + G + E infusion with the responses to infusion of G + E and cortisol alone. During C + G + E administration, plasma glucose (95±2 mg/dl, preinfusion) rose progressively and reached 240±4 mg/dl after 5 h. At 5 h, the increment in plasma glucose produced by C + G + E (144±2 mg/dl) was 136% greater than the sum of the responses to cortisol (3 + 4 mg/dl) and G + E (58±3 mg/dl) ($P < 0.001$) (Fig. 7). The glycemic response to C + G + E was also two- to fourfold greater than the sum of the responses to any double plus single hormone infusion or the sum of the responses to the hormones administered individually ($P < 0.001$) (Fig. 7). As was observed when cortisol was administered with glucagon or epinephrine, infusion of cortisol sustained elevations in glucose production produced by G + E. Glucose production remained 90–125% above preinfusion values for the entire 5-h study period and the increment in glucose production was 70–400% greater than that observed with G + E infusion beyond 60 min ($P < 0.05$–0.001) (Fig. 6). The addition of cortisol to G + E, however, had no effect on glucose clearance or plasma insulin concentration.

β-hydroxybutyrate concentration. β-Hydroxybutyrate levels (0.05±0.01 mM) were not significantly altered by epinephrine administration. Similarly, β-hydroxybutyrate was not increased above base-line values when epinephrine was administered in conjunction with glucagon or when all three counterregulatory hormones were infused simultaneously.

DISCUSSION

The current data demonstrate that the major anti-insulin hormones, glucagon, epinephrine, and cortisol

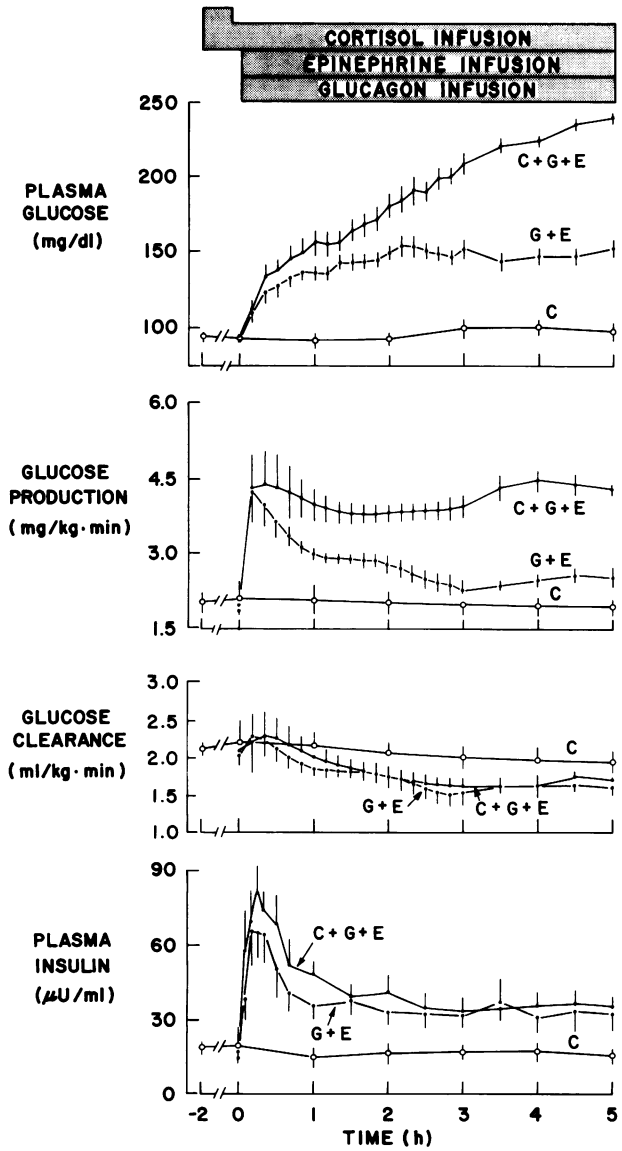

FIGURE 6 Effect of the addition of cortisol (C) on the metabolic response to glucagon plus epinephrine (G + E) infusion.

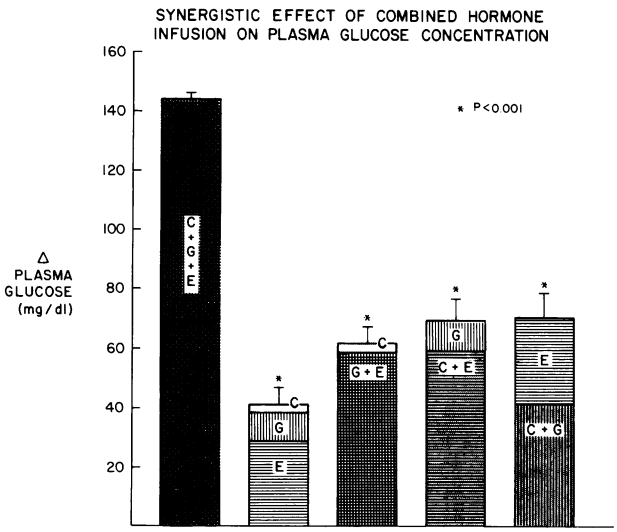

FIGURE 7 Incremental changes in plasma glucose concentration after combined infusion of three counterregulatory hormones as compared with the sum of the responses to the individual hormone infusions or the sum of any combination of double plus single hormone infusion. The rise in plasma glucose produced by the simultaneous infusion of all three hormones (C + G + E) was two- to fourfold greater ($P < 0.001$) than the sum of responses to infusion of cortisol (C), glucagon (G), and epinephrine (E) individually, or the sum of the responses to each combination of two counterregulatory hormones and the respective complementing single hormone infusion.

interact synergistically when physiologic doses of these hormones are acutely administered in the dog. While individual hormone infusions produced only modest changes in blood glucose regulation, simultaneous administration of all three hormones rapidly induced a diabeticlike state characterized by glucose overproduction and plasma glucose levels of 240 mg/dl. Despite earlier evidence that anti-insulin hormones invariably rise in concert during major illness (1–7), little attention has been focused on the importance of hormone-hormone interactions in disorders of glucose homeostasis. Previous studies have been primarily directed toward the influence of basal glucocorticoid secretion (the so-called "permissive action" of glucocorticoids) on the hepatic effects of either glucagon or epinephrine with the adrenalectomized rat model (22–26). The effects of elevations in glucocorticoids on the action of other counterregulatory hormones have been examined only under conditions of chronic steroid treatment and(or) pharmocologic hormone administration (27–28). The present findings extend previous observations by (*a*) providing data on the interactions of glucagon, epinephrine, and cortisol in glucose homeostasis; (*b*) examining these interactions with physiologic quantities of the hormones; and (*c*) evaluating the influence of physiologic cortisol excess on the development of hepatic refractoriness to sustained elevations in plasma glucagon and epinephrine.

The plasma glucagon and cortisol levels achieved during exogenous hormone infusion in the current study were comparable to those reported during severe physiologic stress. Four- to sixfold elevations in plasma glucagon have been observed in the dog in experimental bacterial pneumonia (4) and hypovolemic shock (29); and in man during severe trauma (1–3), cardiogenic shock (30), and diabetic ketoacidosis (31). Two- to threefold increases in plasma cortisol as produced in the current study have been noted after insulin induced hypoglycemia[1] or ACTH administration in the

[1] Saccà, L., and R. S. Sherwin. Unpublished observations.

dog (32). The relatively low plasma cortisol levels observed in the dog (as compared with man) are in agreement with previous data and can be attributed to reduced levels of corticosteroid-binding globulin in the dog (32, 33). Although plasma epinephrine concentration was not directly measured, previous studies which employed larger doses of epinephrine (per kg body wt) have been regarded as physiologic (9, 34). Furthermore, when epinephrine was infused into dogs at twice the current infusion dose,[2] plasma epinephrine rapidly reached plateau levels (800–1,200 pg/ml) which were comparable to those observed in acute insulin-induced hypoglycemia in the dog[3] and in man (35), as well as in diabetic ketoacidosis (36). Thus, it is likely that the concentrations of epinephrine achieved in this study are within the limits observed during physiologic stress.

Infusion of either glucagon or epinephrine alone produced only a transient elevation in glucose production despite ongoing hormone administration. Similar results have been reported with respect to physiologic glucagon infusion in normal dogs and humans (37, 38) and diabetic humans (39), and after epinephrine infusion in the dog (37, 40). Although the elevation in glucose production caused by hyperglucagonemia is transient, recent studies suggest that glucagon may contribute to the maintenance of glucose output at basal levels after the glucagon-stimulated increase in glucose production has waned (41). Inasmuch as plasma glucagon levels were unchanged during epinephrine administration, it is unlikely that the stimulatory effect of epinephrine on glucose production is mediated by augmented glucagon secretion. These findings are in accord with recent studies in the dog, which employed two- to fourfold larger infusion doses of epinephrine (37, 42).

Although the rise in glucose output produced by epinephrine was only 40% of that observed with glucagon, the glycemic response to epinephrine exceeded that of glucagon by 200% ($P < 0.001$) after 3 h (Fig. 2). The mechanism underlying the greater hyperglycemic response to epinephrine becomes apparent when one examines the effect of these hormones on glucose clearance. While infusion of glucagon is accompanied by a 25% increase in glucose clearance, the rate of glucose clearance declined by 20% ($P < 0.02$) during epinephrine administration, thereby accounting for the more pronounced and prolonged effect of the hormone on blood glucose concentration. Inasmuch as the early rise in plasma insulin concentration was comparable in animals that received glucagon and epinephrine, our data suggest that the hyperglycemic actions of glucagon, but not those of epinephrine, are overcome by the stimulatory effects of insulin on glucose clearance. In this regard, in circumstances where the insulin secretory capacity is limited, i.e., diabetes mellitus, the hyperglycemic effects of glucagon are exaggerated as a consequence of the failure of peripheral tissues to dispose of glucose transiently released by the liver (43). Interestingly, despite the transient nature of the stimulatory effect of epinephrine on glucose output, its inhibitory effect on glucose uptake persists for at least 5 h.

When glucagon and epinephrine were infused together, the total increment in plasma glucose observed at 5 h was 50% greater than the sum of the individual infusions ($P < 0.001$). With respect to the mechanism of this synergism, it is noteworthy that the magnitude of the rise in glucose production was no greater than the sum of the individual increments produced by glucagon and epinephrine (Fig. 4). On the other hand, with the combined infusion, the decline in glucose clearance was as great as that observed with epinephrine alone, which contrasts markedly with the rise in glucose clearance observed with glucagon alone. Thus, the addition of epinephrine to glucagon results in an additive increase in glucose output plus an inhibition in fractional glucose uptake. The net result is a more than additive increase in blood glucose. It should be noted, however, that the additive increase in glucose production which accompanied combined glucagon and epinephrine infusion occurred in the face of a twofold greater rise in peripheral insulin levels (Fig. 4). Thus our data do not exclude the possibility that hyperinsulinemia obscured the presence of a synergistic interaction of these hormones on the liver.

The decrease in glucose clearance which accompanied infusion of epinephrine (alone, or in combination with other hormones) occurred despite a transient or sustained two- to fourfold increase in plasma insulin. These data suggest that this effect of epinephrine was not mediated primarily by suppression of insulin secretion. These findings are in accord with in vitro studies which demonstrate that epinephrine directly inhibits glucose uptake in isolated muscle tissue (44). In addition, recent studies indicate that epinephrine administration (0.1 μg/kg · min) reduces the stimulatory effect of exogenous insulin (physiologic doses) on glucose clearance by 65–70% in the conscious dog.[4]

Of particular interest were the effects of cortisol on the response of plasma glucose, and glucose production to glucagon and(or) epinephrine administration. The infusion of cortisol alone (7 h) in physiologic amounts had no effect on plasma glucose, glucose kinetics, plasma insulin, or glucagon concentration (Fig. 2). In contrast to the lack of response to cortisol

[2] Saccà, L., P. Cryer, and R. S. Sherwin. Unpublished observations.

[3] Saccà, L., P. Cryer, and R. S. Sherwin. Unpublished observations.

[4] Saccà, L., and R. S. Sherwin. Unpublished observations.

alone addition of cortisol markedly accentuated the hyperglycemia induced by glucagon and(or) epinephrine. This effect of cortisol was more than additive when combined with either glucagon or epinephrine, or when all three hormones were infused simultaneously (Fig. 7). The augmented glycemic response which accompanied cortisol can be explained primarily on the basis of a change in the stimulatory effect of glucagon and epinephrine on glucose production from a transient to a sustained response (Figs. 5 and 6). Cortisol prevented hepatic refractoriness to sustained elevations in glucagon and(or) epinephrine. In contrast, cortisol had no effect on glucagon- or epinephrine-induced changes in peripheral glucose clearance.

The observation that physiologic elevations in circulating cortisol prolong the actions of hepatic stimulatory hormones differs from the long established concept that glucocorticoids act by permitting other hormones to exert their physiologic effects on glucose production (26). The view that glucocorticoids exert a permissive effect on hormone action is largely based on earlier studies which demonstrate that the in vitro activation of glycogenolysis and gluconeogenesis (with maximal substrate concentrations) by glucagon or epinephrine (in pharmocologic doses) is prevented by adrenalectomy in the rat and restored to normal by glucocorticoid treatment (22–26). These data thus suggest that the presence of sufficient basal concentrations of glucocorticoids are required for stimulation of hepatic glucose release by glucagon or epinephrine. The present findings extend these observations by indicating that acute physiologic cortisol excess in vivo alters the time course of the hepatic response to glucagon and epinephrine, and thus suggest a new mechanism for the diabetogenic actions of adrenal steroids.

In previous studies chronic administration of pharmacologic doses of glucocorticoids resulted in increased basal rates of glucose production and utilization (27, 28). Furthermore, in dogs pretreated with pharmacologic doses of methylprednisolone (3.0–3.5 mg/kg·day) for several days, the glycemic response to pharmacologic doses of glucagon (30–60 ng/kg·min) or epinephrine (0.5 μg/kg·min) was exaggerated 6- to 12-fold as a consequence of a more pronounced rise in hepatic glucose output (27, 28). The current observations underscore the contrasting effects of short term physiologic increments in cortisol, which primarily sustain the stimulatory action of glucagon and epinephrine, and long term pharmacologic doses of glucocorticoids, which increase the magnitude of the response to these hormones. It should be noted, however, that the addition of cortisol increased the initial rise in glucose production produced by glucagon and epinephrine by 47 and 40%, respectively (Fig. 5). While these changes failed to reach statistical significance, our findings do not exclude a possible effect of physiologic elevations in plasma cortisol on the magnitude of the hepatic response to these hormones.

With respect to the mechanism whereby physiologic increments in cortisol prolong the elevations in glucose output caused by glucagon and(or) epinephrine, this action may reflect stimulation of gluconeogenesis as glycogenolysis wanes, maintenance of glycogenolytic rates, or combined stimulation of both processes. An effect on glycogenolysis is suggested by the observation that glucocorticoids increase the synthesis of phosphorylase B as well as its conversion to phosphorylase A, the active form of the enzyme (25). With respect to gluconeogenesis, stimulation by cortisol of key gluconeogenic enzymes (45, 46) and of substrate availability (45, 47) has been reported. Regardless of whether the effect of cortisol involves maintenance of glycogenolysis or stimulation of gluconeogenesis, its action is unlikely to be mediated via changes in glucagon binding to its receptor. Recent studies have shown that rapid onset hepatic refractoriness to glucagon occurs in the absence of altered binding of this hormone to liver membrane receptors (48).

The observations with the combined infusions of glucagon, epinephrine, and cortisol indicate that marked fasting hyperglycemia may be rapidly produced in normal individuals when physiologic increments of multiple counterregulatory hormones occur simultaneously. The glycemic response to infusion of all three hormones was two- to fourfold greater than the sum of the responses to the individual hormone infusions or the sum of any combination of double plus single hormone infusion ($P < 0.001$) (Fig. 7). The marked degree of hyperglycemia in this setting is result of overproduction of glucose which is stimulated by the presence of glucagon and epinephrine, and sustained by an increase in cortisol. Concomitantly, elevations in plasma epinephrine result in an ongoing inhibition of glucose clearance. The synergistic nature of the hormonal interactions suggests that stress hyperglycemia cannot be ascribed to a single hormone, but is a consequence of the combined elevations of several hormones. The current findings indicate that the study of any single counterregulatory hormone underestimates its impact on glucose homeostasis during major stress and thus underscore the important glucoregulatory role of glucagon, epinephrine, and cortisol in clinical situations where these hormones are elevated simultaneously. In this regard, it is possible that growth hormone, which was not examined in this study, but which is elevated in stress (49), may also contribute to the development of hyperglycemia.

The current data are also of interest with regard to the role of epinephrine in modulating insulin secretion. Plasma insulin increased twofold immediately (5–10 min) after epinephrine administration, before a detect-

able increase in plasma glucose (Fig. 2). Later, plasma insulin returned to base line despite the presence of moderate hyperglycemia. These data thus suggest that epinephrine exerts a biphasic influence on insulin secretion in the dog; initially stimulating and subsequently inhibiting insulin release, possibly by its beta and alpha adrenergic stimulatory effects, respectively. In contrast, larger infusion doses of epinephrine in man have been reported to cause an immediate fall in plasma insulin (50). Whether the differences observed between human and dog data reflect species variation or the doses of epinephrine employed remains to be determined. Nevertheless, the failure to observe a rise in insulin in the face of moderate to marked hyperglycemia (Figs. 2, 4, and 6) indicates that the diminution in glucose clearance which accompanies epinephrine infusion (singly or in combination with other hormones) depends, in part, on its inhibitory effects on insulin secretion.

Finally, our data indicate that neither epinephrine alone, nor epinephrine in combination with glucagon or glucagon and cortisol, altered blood levels of β-hydroxybutyrate. Similar results have been reported when glucagon or cortisol are administered individually to normal subjects (51, 52). These findings indicate that in contrast to their effects on blood glucose, physiologic increments of these hormones are, of themselves, insufficient for the development of hyperketonemia in the face of a normal insulin secretory capacity. Our findings are thus in accord with previous data which suggest that insulin deficiency is a prerequisite for the development of hyperketonemia (53). In keeping with these data, blood ketones remained in the normal range in nondiabetic patients who were recovering from extensive trauma, despite consistent elevations in plasma glucose concentration, which in some subjects exceeded 200 mg/dl (1).

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