Adrenergic Mechanisms Contribute to the Late Phase of Hypoglycemic Glucose Counterregulation in Humans by Stimulating Lipolysis

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Abstract

Three studies were performed on nine normal volunteers to assess whether catecholamine-mediated lipolysis contributes to counterregulation to hypoglycemia. In these three studies, insulin was intravenously infused for 8 h (0.30 mU · kg^{-1} · min^{-1} from 0 to 180 min, and 0.40 mU · kg^{-1} · min^{-1} until 480 min). In study I (control study), only insulin was infused; in study II (direct + indirect effects of catecholamines), propranolol and phentolamine were superimposed to insulin and exogenous glucose was infused to reproduce the same plasma glucose (PG) concentration of study I. Study III (indirect effect of catecholamines) was the same as study II, except heparin (0.2 U · kg^{-1} · min^{-1} after 80 min), 10% Intralipid (1 ml · min^{-1} after 160 min) and variable glucose to match PG of study II, were also infused. Glucose production (HGO), glucose utilization (Rd) [3-^{3}H]glucose, and glucose oxidation and lipid oxidation (LO) (indirect calorimetry) were determined. In all three studies, PG decreased from ≈ 4.8 to ≈ 2.9 mmol/liter (P = NS between studies), and plasma glycerol and FFA decreased to a nadir at 120 min. Afterwards, in study I plasma glycerol and FFA increased by ≈ 75% at 480 min, but in study II they remained ≈ 40% lower than in study I, whereas in study III they rebounded as in study I (P = NS). In study II, LO was lower than in study I (1.69±0.13 vs. 3.53±0.19 μmol · kg^{-1} · min^{-1}, P < 0.05); HGO was also lower between 60 and 480 min (7.48±0.57 vs. 11.6±0.35 μmol · kg^{-1} · min^{-1}, P < 0.05), whereas Rd was greater between 210 and 480 min (19±0.38 vs. 11.4±0.34 μmol · kg^{-1} · min^{-1}, respectively, P < 0.05). In study III, LO increased to the values of study I; between 4 and 8 h, HGO increased by ≈ 2.5 μmol · kg^{-1} · min^{-1}, and Rd decreased by ≈ 7 μmol · kg^{-1} · min^{-1} vs. study II. We conclude that, in a late phase of hypoglycemia, the indirect effects of catecholamines (lipolysis mediated) account for at least ≈ 50% of the adrenergic contribution to increased HGO, and ≈ 85% of suppressed Rd. (*J. Clin. Invest.* 1992. 89:2005–2013.) Key words: catecholamines • norepinephrine • epinephrine • free fatty acids • glycerol

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Introduction

The physiological mechanisms of glucose counterregulation have recently been studied in a model of moderate but prolonged hypoglycemia induced by a continuous, low dose infusion of insulin (1–6). This model of prolonged hypoglycemia is relevant to the clinical situation of hypoglycemia in patients with insulinoma (7), and patients with diabetes mellitus treated either with insulin (8, 9) or sulphonylureas (10).

It has been concluded from these studies (11) that in an early phase, glucose counterregulation involves: first, suppression of endogenous insulin secretion, and second, an early but sustained increase in hepatic glucose production, the first two lines of defense against hypoglycemia (1). In a later phase, the third line of defense, suppression of glucose utilization, becomes an important mechanism in preventing a more severe hypoglycemia (1). All counterregulatory hormones, glucagon and adrenaline (rapid hormones) (5, 6), as well as growth hormone and cortisol (slow hormones) (3, 4, 12) contribute to counterregulation, although in different phases and with different mechanisms (11).

In response to insulin-induced hypoglycemia, after initial suppression, plasma FFA, glycerol, and ketone bodies, all rebound above basal levels, whereas plasma alanine decreases with no changes in plasma lactate concentration (1). In theory, changes in one or more of these substrates may contribute to glucose counterregulation. For example, release of lactate, alanine, glycerol, and free fatty acids from peripheral tissues to the liver might sustain gluconeogenesis, the predominant, if not the exclusive, source of glucose produced by the liver during prolonged hypoglycemia (13). In addition, the increase in plasma FFA might suppress peripheral glucose utilization by a mechanism of substrate competition in insulin-sensitive tissues (14, 15), and finally, the increase in plasma ketone bodies might provide an alternative fuel for oxidation by the brain, thus minimizing the effects of neuroglycopenia (16, 17).

Caprio et al. (18) did not find a counterregulatory role of the increase in plasma FFA mediated by adrenergic mechanism during insulin-induced hypoglycemia. However, it is likely that in the Caprio et al. studies (18) only a partial, not a total, adrenergic blockade was achieved, and the unblocked adrenergic effects masqueraded the potential counterregulatory contribution of lipolysis during hypoglycemia.

The present series of studies were undertaken to test the hypothesis that catecholamine-mediated lipolysis plays a physiological role in counterregulation to prolonged insulin hypoglycemia in man. For this purpose, glucose counterregulation was examined in a group of healthy subjects in a model of prolonged hypoglycemia (1, 13), either during suppression or replacement of the increases in plasma glycerol and FFA which

occurs after ≈ 2–2.5 h of hypoglycemia (1). Our results indicate that in a late phase of hypoglycemia, catecholamine-mediated lipolysis contributes to both increased hepatic glucose production and suppressed glucose utilization, thus playing an important counterregulatory role in the prevention of severe hypoglycemia.

Methods

Subjects. Informed consent was obtained from nine healthy volunteers (six males and three females) aged 25±1 yr, all within 10% of their ideal body weight (101±2%, Metropolitan Life Insurance Tables) with no family history of diabetes mellitus or endocrine diseases. For three days before the studies all subjects consumed a weight-maintenance diet containing at least 250 g carbohydrate.

Protocol. Institutional Review Board approval was obtained for these studies. All subjects were admitted to the Clinical Research Center of the Istituto di Medicina Interna e Scienze Endocrine e Metaboliche, University of Perugia, between 6:30 and 7:00 a.m., after fasting overnight (9–10 h). They were placed at bed rest and maintained in the supine position throughout experiments. To obtain arterialized-venous blood samples (19), a hand vein was cannulated in a retrograde position with a 21-gauge butterfly needle, with the hand maintained at 60–65°C in a thermoregulated Plexiglas box. An antecubital vein of the contralateral arm was cannulated with an 18-gauge catheter and used for primed (22 μCi), continuous (0.22 μCi/min) infusion of [3-^{3}H]-glucose (New England Nuclear, Boston, MA) for isotopic determination of glucose production and glucose utilization. Three hours were allowed for isotopic equilibration, after which baseline blood samples were taken. Substrate oxidation and energy expenditure were measured in all subjects by indirect calorimetry (20). 90 min before insulin infusion, a transparent plastic ventilated hood was placed over the subject's head and made airtight around the neck. Air flow and O_2 and CO_2 concentrations in the expired and inspired air were measured by a computerized continuous open-circuit system (Deltatrac; Datex Instruments Co., Helsinki, Finland) (21). Air flow was measured by the air-dilution method, carbon dioxide concentration by a conventional infrared detector, and oxygen concentration by a fast differential paramagnetic oxygen sensor. The monitor has a precision of 2.5% for oxygen consumption and 1.0% for carbon dioxide production. Protein oxidation was estimated from urinary excretion of urea before and during insulin hypoglycemia. After the subjects had adapted to the hood and stabilized their breathing pattern, gas-exchange measurements were taken during a 45-min basal period and were continued throughout the studies.

Three sets of experiments were performed at 1–2-wk intervals. In the first set of experiments (study I), to induce moderate hypoglycemia, insulin (Actrapid HM U-40; Novo Research Institute, Copenhagen, Denmark), diluted to 1 U/ml in 100 ml of 0.9% NaCl containing 2 ml of the subject's blood, was intravenously infused at the rate of 0.30 $mU \cdot kg^{-1} \cdot min^{-1}$ from 0 to 180 min and 0.40 $mU \cdot kg^{-1} \cdot min^{-1}$ from 180 to 480 min with a syringe pump (Harvard Apparatus Co., Inc., The Ealing Corp., South Natick, MA). This intravenous rate of insulin infusion was chosen to reproduce the mild hyperinsulinemia observed in previous studies during subcutaneous insulin infusion in normal man (1–6), and to simulate the type of hypoglycemia that may develop in diabetic patients undergoing intensive insulin therapy (8, 9, 22–25).

In the second set of experiments (study II), insulin was infused as in study I, but propranolol (priming dose 3 mg over 3 min, followed by 0.1 $mg \cdot min^{-1}$ continuous infusion) and phentolamine (priming dose 10 mg over 3 min, followed by 0.5 $mg \cdot min^{-1}$ continuous infusion) were infused from 0 to 480 min to block the catecholamine mediated rebound increase in FFA which occurred in study I after 120 min. Because we anticipated from previous studies (6) that combined α-,β-adrenergic blockade would result in impaired glucose counterregulation and more severe hypoglycemia, in study II exogenous glucose was infused when needed to maintain plasma glucose concentration at the values observed in study I by means of a modified glucose clamp technique (3–6, 26) based on plasma glucose concentration measured every 2.5–5 min. This enabled us to avoid the confounding effects of lower plasma glucose concentrations in study II as compared to study I on glucose fluxes by a mass action effect (27); to prevent greater counterregulatory hormone responses because of severe hypoglycemia (28); and also avoid the discomforts and potential risks of severe hypoglycemia.

In the third set of experiments (study III), insulin, propranolol, and phentolamine were infused as in study II, and when needed, exogenous (cold) glucose was infused to maintain the same plasma glucose concentrations of study II. In addition, in study III heparin (0.2 $mU \cdot kg^{-1} \cdot min^{-1}$ from 80 to 480 min) and a triglyceride emulsion (10% Intralipid; Kabi Pharmacia, Stockholm, Sweden, 1 $ml \cdot min^{-1}$ from 160 to 480 min) were infused to reproduce the rebound increase in glycerol and FFA observed in study I after 120 min. In the 10% Intralipid the total amount of glycerol deriving from triglycerides and phospholipids, with added glycerol was ≈ 330 mM. Thus, in study III the estimated rate of glycerol infusion was ≈ 4.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ under the assumption that the infused triglycerides and phospholipids were fully hydrolized. The above rates of infusion of heparin and 10% Intralipid in study III were chosen after several pilot experiments based on the "trial and error" method.

Analyses. Blood samples were collected at 30-min intervals and assayed for glucose (Glucose Analyzer; Beckman Instruments, Fullerton, CA), glucose specific activity (29), insulin (30), C-peptide (31), glucagon (32), cortisol (33), growth hormone (34), epinephrine (35), free fatty acids by an enzymatic colororimetric method (NEFA C test kit; Wako Chemicals GmbH, Neuss, Germany), 3-β-OH-butyrate (36), glycerol (36), and lactate (36) by previously described methods. In initial pilot experiments with heparin-Intralipid infusion, it was found that in vitro lipolysis (i.e., in tubes where whole blood was collected and allowed to clot for 1–2 h at room temperature) caused a false increase in plasma FFA up to ≈ 70%. Therefore, to prevent the pitfall of in vitro lipolysis which might have led to an underestimation in the rate of heparin-Intralipid infusion and underreplacement of FFA and glycerol in study III, in all studies blood (2 ml) for FFA determination was collected in tubes containing 50 μl of the lipoproteinlipase inhibitor diethyl-*p*-nitrophenyl-phosphate (Paraoxon; Sigma Chemical Co., St. Louis, MO) diluted to 0.04% in diethyl ether, as previously described (37). Urine was collected from the onset to the end of each study period to determine nitrogen excretion using the Kjeldahl method (38). Oxidation rates for carbohydrate, fat, and protein were calculated from the measured O_2 consumption, CO_2 production, and urinary nitrogen excretion (39). Rates of glucose production and uptake in the steady state were computed using the isotope dilution equation. Glucose specific activity reached an apparent steady state in each subject before insulin infusion. After insulin infusion, the rates of glucose appearance (production) and disappearance (utilization) were calculated using the non-steady state equations of De Bodo et al. (40), and were "smoothed" according to the method of Miles et al. (41). Data in text and figures are given as mean±SE, and the statistical significance was evaluated using analysis of variance corrected for repeated measures (42).

Results

Plasma insulin and C-peptide (Fig. 1). In the control experiments (study I), plasma insulin concentration increased from a baseline of 56±6.6 pmol/liter to 153±12 pmol/liter by 180 min (insulin infusion rate 0.3 $mU \cdot kg^{-1} \cdot min^{-1}$) and to 180±14 pmol/liter between 180 and 480 min (insulin infusion rate 0.4

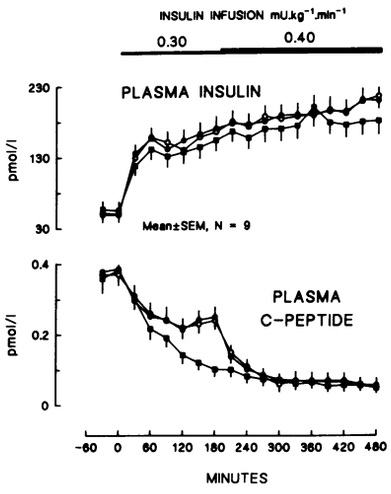

Figure 1. Plasma insulin and C-peptide concentration in studies I–III. ■, Saline; ●, α,β-blockade + glucose; ○, α,β-blockade + glucose + heparin + intralipid.

mU · kg^{-1} · min^{-1}). In the α-,β-blockade (study II) and heparin-Intralipid experiments (study III), the plasma insulin concentrations were superimposable to those of the control experiments.

Plasma C-peptide concentration progressively decreased in all three studies, and was suppressed by > 85% after 300 min as compared to the baseline values. However, in studies II and III, plasma C-peptide concentration was less suppressed, from 90 to 210 min as compared with study I ($P < 0.05$), a consequence of deinhibition by α-,β-blockade of suppressed pancreatic β-cell secretion by catecholamines during hypoglycemia, as previously reported (6). Such an increase in plasma C-peptide was not paralleled by an increased peripheral plasma insulin concentration, probably because the increase in C-peptide was transient (≈ 90 min) and quantitatively modest (≈ 0.1 pmol/liter).

Plasma glucose concentrations, rates of exogenous glucose infusion, and rates of glucose production and utilization (Fig. 2). In the control experiments (study I), plasma glucose concentration progressively decreased from a baseline value of 4.8±0.08 mmol/liter to 2.9±0.12 mmol/liter at 480 min. In the α-,β-blockade (study II) and heparin-Intralipid experiments (study III), plasma glucose concentrations were maintained superimposable to those of control experiments (P = NS) by means of infusing exogenous glucose at variable rate between 30 and 480 min. However, in the experiments with heparin-Intralipid in study III, the mean rate of exogenous glucose infusion needed to maintain plasma glucose concentration superimposable to that of the control experiments in study I, was only ≈ 40% compared to the α-,β-blockade experiments of study II (4.82±0.44 vs. 12.0±1.3 μmol · kg^{-1} · min^{-1}, respectively, 240–480 min, $P < 0.05$).

In the control experiments, after an initial suppression at 60 min, the rate of hepatic glucose production increased from a baseline of 11.6±0.7 μmol · kg^{-1} · min^{-1} to a peak value of 14.2±0.90 μmol · kg^{-1} · min^{-1} at 180 min ($P < 0.05$). Afterwards, glucose production slowly decreased to 10.0±0.53 μmol · kg^{-1} · min^{-1} at 480 min. In the α-,β-blockade experiments, the increased rate of glucose production was less than in the control experiments, and overall was ≈ 30% lower than in the control experiments after 30 min (7.48±0.57 vs. 11.6±0.35 μmol · kg^{-1} · min^{-1}, respectively, 60–480 min, $P < 0.05$). In the heparin-Intralipid experiments, the rate of hepatic glucose production was initially as suppressed as in the α-,β-blockade experiments until 210 min. Then, hepatic glucose production was less suppressed, and was ≈ 43% greater than in the α-,β-blockade experiments between 240 and 480 min (8.35±0.22 vs. 5.85±0.35 μmol · kg^{-1} · min^{-1}, respectively, $P < 0.05$).

In the control experiments, the rate of peripheral glucose utilization transiently increased from a baseline value of 11.6±0.71 μmol · kg^{-1} · min^{-1} to 14.4±0.8 μmol · kg^{-1} · min^{-1} at 180 min ($P < 0.05$). Afterwards, glucose utilization progressively decreased to 10.2±0.6 μmol · kg^{-1} · min^{-1} at 480 min. In the α-,β-blockade experiments, glucose utilization increased more than in the control experiments between 180 and 210 min (16.2±1.2 vs. 13.4±0.8 μmol · kg^{-1} · min^{-1}, respectively, $P < 0.05$). However, in the heparin-Intralipid experiments, after 210 min the rate of glucose utilization was more suppressed than in the α-,β-blockade experiments and overall was ≈ 36% lower as compared to the α-,β-blockade experiments (12.3±0.53 vs. 19.2±0.36 μmol · kg^{-1} · min^{-1}, respectively, 240–480 min, $P < 0.05$).

Plasma counterregulatory hormone concentrations (Fig. 3). In the control experiments, plasma counterregulatory hormones increased to an approximate steady state between 240 and 480 min; by 480 min, glucagon from 101±18 to 170±15 pg/ml; epinephrine from 54±9 to 375±74 pg/ml; growth hormone from 2.5±1.2 to 9.2±1.7 ng/ml, and cortisol from 11.5±0.9 to 15.2±1 μg/dl. In the α-,β-blockade experiments, the values of plasma glucagon and cortisol were no different when compared to those of the control experiments, whereas plasma epinephrine increased approximately twofold by the end of the study (893±109 vs. 375±74 pg/ml at 480 min, study II vs. study I, respectively, $P < 0.05$), also growth hormone increased approximately twofold (18±1.9 vs. 9.2±1.7 ng/ml at 480 min, study II vs. study I, $P < 0.05$). Finally, in the heparin-Intralipid experiments, plasma glucagon, epinephrine and cor-

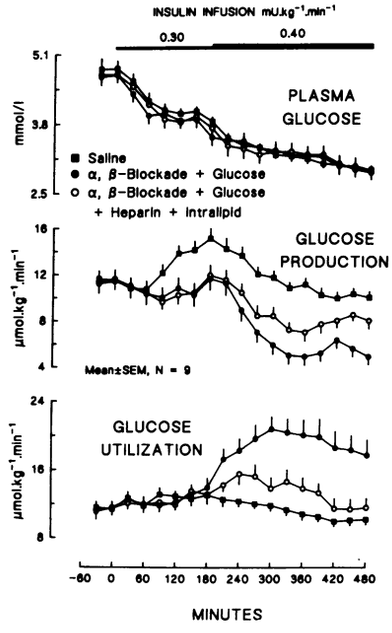

Figure 2. Plasma glucose concentrations, rates of endogenous (hepatic) glucose production, and peripheral glucose utilization in studies I–III. In α-,β-blockade experiments (study II, *full circles*) and heparin-Intralipid experiments (study III, *empty circles*), an infusion of glucose at variable rate was initiated after 30 min and continued throughout 480 min to maintain plasma glucose concentrations superimposable to those of control experiments (study I, saline, *full squares*).

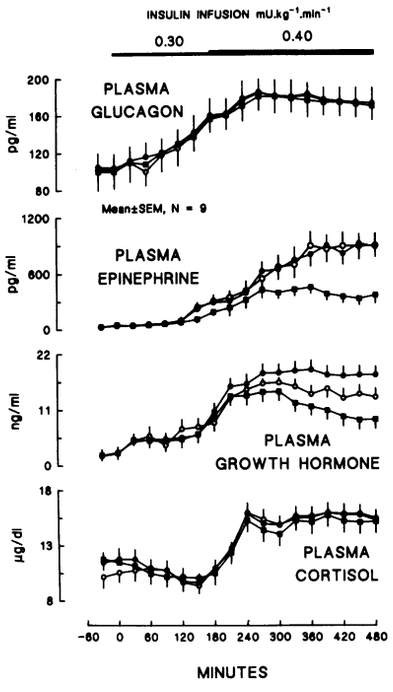

Figure 3. Plasma counterregulatory hormone concentrations in studies I–III. ■, Saline; ●, α,β-blockade + glucose; ○, α,β-blockade + glucose + heparin + intralipid.

tisol responses were superimposable to those of the α-,β-blockade experiments, whereas growth hormone responses were ≈ 25% lower than those of the α-,β-blockade experiments (13.6±1.5 vs. 18±1.9 pg/ml at 480 min, respectively, $P < 0.05$).

Plasma glycerol and FFA concentrations, rates of lipid and glucose oxidation (Fig. 4), and β-OH-butyrate and lactate concentrations. In the control experiments, plasma glycerol decreased soon after initiation of insulin infusion from baseline values of 0.042±0.005 mM to a nadir of 0.025±0.002 mM at 120 min. Similarly, plasma FFA decreased from 0.43±0.05 mM to a nadir of 0.155±0.022 mM at 120 min. Afterwards, both plasma glycerol and FFA progressively increased to 0.072±0.009 mM and 0.782±0.05 mM by 480 min (glycerol and FFA, respectively, $P < 0.05$). In the α-,β-blockade experiments, plasma glycerol and FFA were suppressed more than in the control experiments with nadir values between 180 and 240 min (plasma glycerol 0.025±0.002 mM, plasma FFA 0.058±0.011 mM). Afterwards, both plasma glycerol and FFA increased less than in the control studies (by 480 min plasma glycerol 0.042±0.06 mM, plasma FFA 0.355±0.026 mM, $P < 0.05$). In the heparin-Intralipid experiments, plasma glycerol and FFA were initially suppressed, and subsequently rebounded to values superimposable to those of the control studies (P = NS).

Plasma β-OH-butyrate followed the pattern of plasma glycerol and FFA (data not shown). In the control experiments, plasma β-OH-butyrate decreased from a baseline value of 0.171±0.02 mM to a nadir of 0.072±0.01 mM at 120 min, then progressively increased to 0.56±0.07 mM by 480 min. In the α-,β-blockade experiments, plasma β-OH-butyrate concentration was suppressed more (nadir value at 240 min of 0.48±0.04 mM), and increased less than in the control experiments (by 480 min 0.21±0.09 mM). In the heparin-Intralipid experiments, the rebound in plasma β-OH-butyrate after 120 min was superimposable to that of the control studies (0.59±0.09 mM by 480 min, P = NS vs. control study).

In the control experiments, plasma lactate (baseline 0.97±0.06 mM) did not change (data not shown). In the α-,β-blockade experiments, plasma lactate progressively decreased after 270 min and was 0.82±0.05 mM at 480 min, $P < 0.05$ as compared to the control experiments (0.95±0.06 mM). In the heparin-Intralipid experiments, plasma lactate concentration was superimposable to that of the α-,β-blockade experiments.

The pattern of lipid oxidation was parallel to plasma glycerol and FFA concentrations. In the control experiments, the rate of lipid oxidation decreased from a baseline value of 2.89±0.31 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to a nadir of 1.53±0.21 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 120 min. Afterwards, lipid oxidation progressively increased to 4.10±0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ by 480 min. In the α-,β-blockade experiments, lipid oxidation was suppressed more than in the control study, and increased ≈ 40% less by 480 min (2.47±0.32 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$ vs. control study). In the heparin-Intralipid experiments, lipid oxidation was superimposable to that of the control studies.

Glucose oxidation increased in the control experiments from a baseline value of 7.56±1.11 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to a peak of 12.5±1.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 180 min, and after progressively decreased to 6.85±0.66 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ by 480 min. In the α-,β-blockade experiments, glucose oxidation was not suppressed after 180 min, and remained greater than in the control experiments (by 480 min 11.6±0.94 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, $P < 0.05$). In the heparin-Intralipid experiments, after 180 min the rate of glucose oxidation was suppressed more than in the α-,β-blockade experiments, but less than in the control experiments (10±0.52, 12.8±0.25, and 8.84±0.64 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, 210–480 min, $P < 0.05$).

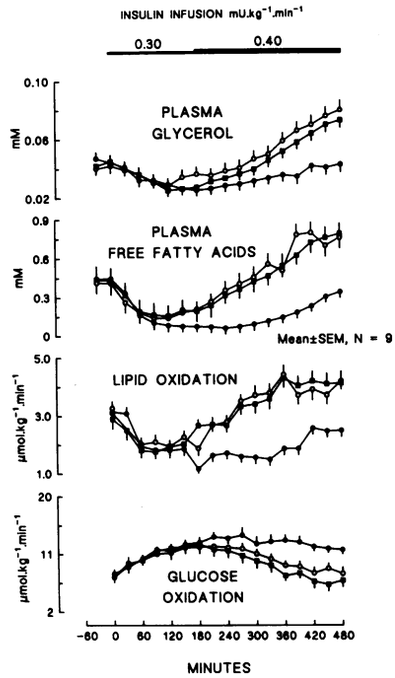

Figure 4. Plasma glycerol and FFA concentrations, and rates of lipid and glucose oxidation in studies I–III. ■, Saline; ●, α,β-blockade + glucose; ○, α,β-blockade + glucose + heparin + intralipid.

Correlations between rates of lipid oxidation and glucose fluxes (Fig. 5). In the heparin-Intralipid experiments, the percent increase in lipid oxidation between 150 and 480 min significantly correlated with the percent decrease in the glucose infusion rate required to reproduce the same plasma glucose concentration of the α-,β-blockade experiments (r = 0.95), and with the percent increase in hepatic glucose production (r = 0.88), as well as the percent decrease in glucose utilization (r = 0.98) (all $P < 0.05$). Thus, the greater the availability of the substrates FFA and glycerol, the greater the rate of lipid oxidation, and the greater the increase in hepatic glucose production and the suppression of glucose utilization during the late phase of insulin-induced hypoglycemia.

Discussion

The present studies reaffirm the recent finding (6) that adrenergic mechanisms do play a key role in the prevention of severe hypoglycemia both at a very early as well as late phase of glucose counterregulation in response to intravenous insulin infusion, and for the first time demonstrate that in a late phase of hypoglycemia the adrenergic contribution to glucose counterregulation is partially dependent on stimulation of lipolysis. Stimulation of lipolysis by adrenergic mechanisms after 180–210 min of counterregulation, contributed in increasing glucose production by $\approx$ 50% and suppressing peripheral glucose utilization by $\approx$ 85% as compared to experiments in which lipolysis was almost fully blocked. This means that in a late phase of hypoglycemia, the activation of lipolysis by catecholamines is a powerful counterregulatory mechanism. With the exception of the increase in hepatic glucose production in response to severe hypoglycemia per se, i.e., independent of hormonal, neural mechanisms, and nonglucose substrates, the so-called hepatic autoregulation (43), the present study is, to the best of our knowledge, the first demonstration of a direct counterregulatory mechanism mediated by nonglucose substrates (FFA and glycerol) in defense against hypoglycemia in man.

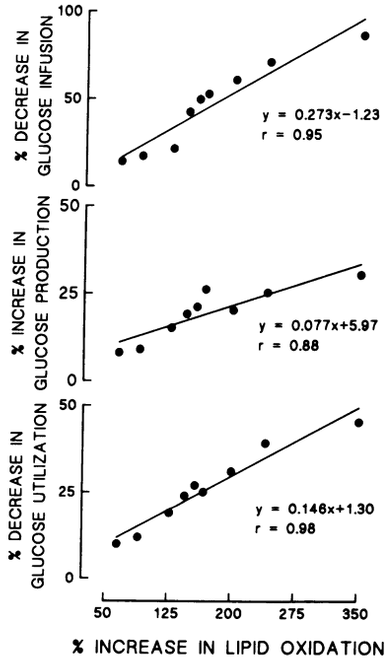

Figure 5. Correlations between the percent increase in lipid oxidation after 150 min in the heparin-Intralipid experiments (study III) as compared to the α-,β-blockade experiments (study II) and, *top panel,* the decrease in the rate of exogenous glucose infusion required to reproduce in study III the same plasma glucose concentration of study II; *middle panel,* the percent increase in the rate of hepatic glucose production in study III as compared to study II; *bottom panel,* the percent decrease in the rate of peripheral glucose utilization in study III as compared to study II.

Regarding the contribution of stimulated lipolysis to increased hepatic glucose production in the present experiments, it is likely that both the increased glycerol and FFA fluxes to the liver contributed to sustain primarily gluconeogenesis, and not glycogenolysis, the former being the predominant, if not the exclusive, mechanism of increased glucose production by the liver after $\approx$ 3 h of hypoglycemia (13). Normally, glycerol accounts for $\approx$ 3% of glucose released from the liver in the postabsorptive state (44), whereas gluconeogenesis is estimated to contribute by one-third to overall glucose production. In addition, gluconeogenesis accounts for $\approx$ 90% of overall hepatic glucose output in a model of prolonged hypoglycemia (13) identical to that of the present studies. Thus, it is likely that glycerol-derived gluconeogenesis contributed to at least 10% of overall hepatic glucose output in the late phase of the present experiments, and possibly even more, because plasma glycerol concentration increased above baseline values after 330 min, and it is well known that the supply of glycerol originating from lipolysis is the major factor regulating glycerol conversion to glucose (45, 46). In this regard, the estimate of the rate of glycerol infusion in the experiments of study III in which heparin and 10% Intralipid were infused, yielded a figure of $\approx$ 4.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ assuming a complete hydrolysis of the infused fat emulsion into FFA and glycerol in plasma. This rate is almost three times greater than the basal turnover rate of glycerol in man ($\approx$ 1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (44). However, because in the late phase of hypoglycemia, plasma glycerol concentration increases by approximately three times as compared to its early nadir (Fig. 4, study I), it is likely that rates of glycerol appearance, two to three times greater than the baseline are required to produce such a sustained increase in plasma glycerol concentration by the end of studies. In fact, using such rates of glycerol infusion, in the experiments of study III, the plasma glycerol concentrations very closely matched those of the control experiments of study I. Taken together, these considerations support the concept that the increase in hepatic glucose production observed in the present heparin-Intralipid experiments of study III as compared to that of the α-,β-blockade of study II was due to physiological, and not pharmacological glycerol replacement, closely mimicking the catecholamine stimulated lipolysis of control study I.

It is likely that not only glycerol, but also FFA contributed to the increase in the rate of hepatic glucose production observed in the heparin-Intralipid as compared to the α-,β-blockade experiments of the present studies. Indirect support for an interaction between FFA and hepatic glucose production has been derived from studies demonstrating a positive correlation between FFA or lipid oxidation and rates of hepatic glucose production (47–49). In fact, FFA generated acetyl-CoA within the liver, stimulates pyruvate carboxylase, the first committed step of the gluconeogenic pathway (50). By this mechanism, FFA can increase gluconeogenesis (47, 51–53) and hepatic glucose production (54–57). In the present studies, because of prolonged hypoglycemia the liver was exposed to the gluconeogenetic effects of glucagon (58), cortisol (58), and most likely also growth hormone (59). It is possible that under these conditions even the apparently modest increase in plasma FFA ($\approx$ 0.5 mM) in the present experiments accelerated gluconeogenesis and increased hepatic glucose produc-

tion. This concept is supported in the present studies by the correlation found between the increase in lipid oxidation (primarily FFA) and hepatic glucose production after activation of lipolysis by counterregulation in the heparin-Intralipid experiments as compared to the α-,β-blockade experiments (Fig. 5, *middle panel*).

Although the design of the present experiments does not allow one to distinguish between the relative contribution of glycerol and FFA to the increased hepatic glucose production in the late phase of hypoglycemia, it should be considered that the release of both these substrates from adipose tissue is driven by adrenergic mechanisms. Adrenergic mechanisms may sustain gluconeogenesis via two mechanisms, i.e., by providing greater flux of gluconeogenetic substrates (such as lactate, glycerol, alanine, etc.) and FFA from peripheral tissues, and by increasing the efficiency of the intrahepatic conversion of these substrates to glucose (60). It is possible that the increase in hepatic glucose production observed in the present heparin-Intralipid experiments of study III, reflect increased gluconeogenesis secondary to greater supply of substrates and FFA to the liver. However, because the effects of catecholamines were blocked, it is also possible that the overall efficiency of gluconeogenesis was reduced. Thus, despite a normal supply of glycerol and FFA to the liver, hepatic glucose production was not normalized in the heparin-Intralipid experiments of study III.

Regarding the suppression of glucose utilization by catecholamine-stimulated lipolysis, the present studies provide a direct evidence that this effect is secondary to a substrate competition effect between FFA and glucose, both in the oxidation and in the utilization processes of peripheral tissues. This finding is also consistent with Randle's glucose-fatty acid hypothesis (61, 62). Because the competition between FFA and glucose utilization becomes evident only when glucose uptake by peripheral tissues is increased by hyperinsulinemia (14, 15), and because the muscle is the primary site of increased glucose utilization promoted by insulin (63), it is assumed that the in vivo effects of elevated FFA in suppressing glucose oxidation and utilization take place at the muscle level.

In the present studies, the rates of glucose oxidation decreased, whereas those of lipid oxidation increased after stimulation of lipolysis after 240 min (Fig. 4), as previously reported (18, 64). In addition, the increase in the rates of lipid oxidation was inversely correlated with the decrease in the rates of glucose utilization in the heparin-Intralipid as compared to the α-,β-blockade experiments (Fig. 5, *lower panel*). However, these data must be interpreted with some caution. During prolonged hypoglycemia (13), increased gluconeogenesis from amino acids increases nitrogen production, whereas increased production of ketone bodies may influence gas exchange (39). This introduces a theoretical limitation in the calculation of accurate partitioning of whole body oxidative metabolism into its components of net carbohydrate, fat, and protein oxidation (39). This would cause an underestimation of glucose oxidation directly proportional to the amount of de novo synthetized glucose from alanine, as well as an underestimation of lipid oxidation (39). Therefore, the decrease in carbohydrate oxidation rates observed in the present experiments after 240 min are, if anything, an overestimation of the suppression of the rates of glucose oxidation by increased lipid oxidation. In this regard, the rate of glucose oxidation would be underestimated less in the α-,β-blockade experiments, because the adrenergic blockade would suppress the catecholamine-induced gluconeogenesis (60). On the other hand, in the heparin-Intralipid experiments, FFA-driven gluconeogenesis would again result in a greater underestimation of glucose oxidation as compared to the α-,β-blockade experiments, although quantitatively lower than in the control experiments where rates of hepatic glucose production (and most likely gluconeogenesis) are greater. Although these methodological limitations make it difficult to estimate quantitatively the rates of glucose oxidation, it is likely that the suppression of glucose oxidation observed in the present studies in a late phase of hypoglycemia is not the consequence of a direct adrenergic response. In fact, Wolfe and Shaw (65) demonstrated in the dog that epinephrine does not suppress glucose oxidation if glucose flux, FFA availability, and glucoregulatory hormones are kept constant. Taken together, the qualitative changes of glucose oxidation observed in the present studies and, more importantly, the changes in the rates of glucose utilization are nevertheless consistent with the substrate competition hypothesis between glucose and FFA. The results of the present studies suggest that the glucose-FFA cycle (61, 62) is a physiological mechanism which induces peripheral (muscular) insulin resistance in a late phase of hypoglycemic glucose counterregulation. Thus, the glucose-FFA cycle is operative not only in obesity (66) and type 2 diabetes mellitus (67, 68), but also intervenes as a physiological mechanism in defense against hypoglycemia.

It is interesting to note that in previous studies (14, 15), evidence of a glucose-FFA substrate competition effect in vivo in peripheral tissues (primarily muscle) has been demonstrated for supraphysiological increases in plasma FFA in the order of 1.5–2.5 mM above those of control studies. In contrast, in the present studies, the substrate competition effect between FFA and glucose for peripheral glucose utilization is clearly evident with only a difference of $\approx$ 0.5 mM in plasma FFA concentration. This indicates that under conditions of hypoglycemic glucose counterregulation, peripheral tissues (primarily muscle) are exquisitely sensitive to minor changes in plasma FFA availability, and that plasma FFA concentration might be a much more important regulatory mechanism in the physiology of muscle oxidation and utilization of glucose than currently appreciated.

In theory, the rebound increase in β-OH-butyrate which was parallel to FFA in the present studies, might have competed for glucose utilization in the brain, thus contributing to suppress the rate of glucose utilization (16). However, it is unlikely that this was the case because the counterregulatory hormonal responses observed during suppression (α-,β-blockade experiments) and replacement (heparin-Intralipid experiments) of plasma β-OH-butyrate concentration were almost identical. As recently reported (17), if plasma β-OH-butyrate was utilized as a fuel instead of glucose, a lower counterregulatory hormone response would have been observed in the latter as compared to the former experiments because of compensation of neuroglycopenia by oxidation of ketone bodies.

In their recent study, Caprio et al. (18) did not find a counterregulatory role of catecholamine-stimulated increase in FFA in a model of hypoglycemia similar to that of the present experiments. Probably the shorter duration of their study ($\approx$ 240 vs. $\approx$ 480 min of the present studies) and the lower peripheral plasma insulin concentration achieved ($\approx$ 140 vs. $\approx$ 180 pmol/liter of the present experiments) accounted for the lack of

β-adrenergic effects on the extrahepatic mechanisms of glucose utilization (18). However, in their study it is unclear as to why β-blockade did not result in impaired hepatic glucose counterregulation, since catecholamines do play a key role in increasing hepatic glucose production in defense against hypoglycemia at a very early phase (6). The only difference between the Caprio et al. study (18) and the studies in which catecholamines have been found to be important (6, present study) is that in the Caprio et al. study (18) only β-blockade was carried out, whereas in previous studies (6) and in the present studies, combined α-,β-blockade was performed. Isolated β-blockade during hypoglycemia may indirectly enhance α-adrenergic mediated effects (69, 70), such as suppression of endogenous insulin secretion more rapidly than in the control study (6). Thus, it is possible that in the study by Caprio et al. (18) a lower portal hyperinsulinemia during isolated β-blockade as compared to the control study masqueraded the contribution of catecholamines on hepatic glucose counterregulation.

In interpreting the results of the present studies, the difference in plasma growth hormone concentration between the α-,β-blockade and the heparin-Intralipid experiments should be taken into account. First, plasma growth hormone concentration was greater in the α-,β-blockade and heparin-Intralipid experiments as compared to control experiments because of deinhibition of the β-adrenergic tone by propranolol (71) as previously reported (6, 29, 72). Second, in the late phase of heparin-Intralipid experiments, growth hormone was lower than in the α-,β-blockade experiments, most likely because elevated plasma FFA suppress growth hormone secretion (73, 74). However, if anything, this would underestimate the contribution of stimulated lipolysis to counterregulation, because the subjects should be more insulin sensitive in the heparin-Intralipid than in the α-,β-blockade experiments because of the less increased plasma GH responses.

The results of the present studies expand our understanding of the counterregulatory mechanisms of catecholamines in defense against hypoglycemia in man. In an early phase, catecholamines suppress endogenous insulin secretion (Fig. 1), and contribute to increase hepatic glucose production after 30 min (Fig. 2), most likely by stimulating glycogenolysis. Later, they continue to increase hepatic glucose production (here gluconeogenesis is probably the major source), but also suppress peripheral glucose utilization. Clutter et al. (75) have named the catecholamine-induced suppression of endogenous insulin secretion as an indirect effect, and the increased hepatic glucose production and the suppressed glucose utilization as direct counterregulatory effects of catecholamines. The present studies reaffirm that catecholamines suppress endogenous insulin secretion in an early phase of hypoglycemia (Fig. 1), as recently reported (6). In addition, the present studies for the first time indicate that the indirect counterregulatory effects of catecholamines include stimulation of lipolysis. This mechanism plays an important counterregulatory role because it sustains hepatic glucose production and suppresses peripheral glucose utilization, ultimately preventing more severe hypoglycemia.

The present studies also allow one to speculate on the quantitative role of the indirect counterregulatory effects of adrenergic mechanisms mediated by accelerated lipolysis, as compared to its direct effects (Table I). In the late phase of hypoglycemia (240–480 min), the total contribution of catecholamines (direct + indirect effects) to hepatic glucose production increased

Table I. Contribution of the Effects of Catecholamines to Increased Glucose Production and Suppressed Glucose Utilization in the Late Phase (240–480 min) of Insulin Hypoglycemia

	Study I	Study II	Study III
Hepatic glucose production ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	10.9±0.29	5.85±0.35	8.35±0.22
Percentage of control study	100%	≈54%	≈77%
Glucose utilization ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	11.1±0.29	19.2±0.36	12.3±0.53
Percentage of control study	58%	100%	64%

The overall (direct + indirect) effects are estimated as difference between the α-,β-blockade studies (study II) and the control studies (study I). The indirect effects are estimated as difference between α-,β-blockade + heparin + Intralipid studies (study III) and α-,β-blockade studies (study II).

by overall counterregulation, calculated as the difference between study I and study II, was ≈ 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, i.e., ≈ 46% of the hepatic glucose production of study I (saline). The indirect effects of adrenergic-mediated lipolysis accounted for ≈ 2.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, i.e., ≈ 50% of the total adrenergic contribution to hepatic glucose production. Regarding glucose utilization, overall catecholamines suppressed glucose utilization by ≈ 8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (difference between study II and study I), i.e., they reduced glucose utilization by ≈ 42% (direct + indirect effects). Catecholamine-mediated lipolysis (difference between studies II and III) accounted for by ≈ 7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, i.e., ≈ 85% of the catecholamine effects on glucose utilization are indirect. Thus, the contribution of stimulated lipolysis to hypoglycemic glucose counterregulation is relevant both to the liver as well as to the muscle, but it appears to be quantitatively more important for the latter rather than for the former.

It should be pointed out that these indirect counterregulatory effects of adrenergic mechanisms mediated by accelerated lipolysis became important only in a late phase of hypoglycemia, i.e., after three hours. Thus, these indirect mechanisms may contribute in preventing a more severe hypoglycemia during prolonged, not short-term hypoglycemia (1). In this regard, it is interesting to note that other counterregulatory hormones such as glucagon, cortisol, and growth hormone failed to fully compensate for the direct effects of catecholamine at the end of study III of the present experiments where plasma glycerol and FFA were increased by the heparin-Intralipid infusion. This observation is consistent with our previous finding of lack of reciprocal compensation between counterregulatory hormones, when one of them fails to increase (3–6). It is also tempting to speculate on the possibility that activation of adrenergic mechanisms under conditions different from hypoglycemia for example, stress might cause hyperglycemia at least in part by the indirect mechanism of stimulated lipolysis demonstrated in the present experiments.

The stimulated lipolysis observed in the present experiments after approximately three hours of activated counterregulation, was largely the result of increased secretion of catecholamines. However, the late increase in plasma glycerol and FFA concentrations after five to six hours despite adrenergic α-,β-blockade in study II, indicates that most likely other coun-

terregulatory hormones such as growth hormone (3) and cortisol (4) contributed to the late FFA and glycerol rebound. Therefore, it is possible that not only catecholamines, but also growth hormone and cortisol, which possess lipolytic actions in hypoglycemic counterregulation (3, 4), increase hepatic glucose production and suppress peripheral glucose utilization at least in part by indirect mechanisms, i.e., stimulated lipolysis.

In summary, the present studies indicate that adrenergic mechanisms counterregulate hypoglycemia partially by indirect mechanisms, i.e., by stimulating lipolysis and increasing the glycerol and FFA fluxes to the liver and muscle. This increases hepatic glucose production, suppresses peripheral glucose utilization, and ultimately prevents more severe hypoglycemia. Further studies are needed to establish whether such a stimulated lipolysis during hypoglycemia contributes to glucose counterregulation not only under the conditions of α-,β-blockade, as in the present study, but also when the hepatic and extrahepatic effects of catecholamines (and other counterregulatory hormone) are not inhibited.

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