The Role of Adrenergic Mechanisms in the Substrate and Hormonal Response to Insulin-Induced Hypoglycemia in Man

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A B S T R A C T   Sequential determinations of glucose outflow and inflow, and rates of gluconeogenesis from alanine, before, during and after insulin-induced hypoglycemia were obtained in relation to alterations in circulating epinephrine, norepinephrine, glucagon, cortisol, and growth hormone in six normal subjects. Insulin decreased the mean ($\pm$SEM) plasma glucose from 89$\pm$3 to 39$\pm$2 mg/dl 25 min after injection, but this decline ceased despite serum insulin levels of 153$\pm$22 μU/ml. Before insulin, glucose inflow and outflow were constant, averaging 125.3$\pm$7.1 mg/kg per h. 15 min after insulin, mean glucose outflow increased threefold, but then decreased at 25 min, reaching a rate 15% less than the preinsulin rate. Glucose inflow decreased 80% 15 min after insulin, but increased at 25 min, reaching a maximum of twice the basal rate. Gluconeogenesis from alanine decreased 68% 15 min after insulin, but returned to preinsulin rates at 25 min, and remained constant for the next 25 min, after which it increased linearly. A fourfold increase in mean plasma epinephrine was found 20 min after insulin, with maximal levels 50 times basal. Plasma norepinephrine concentrations first increased significantly at 25 min after insulin, whereas significantly increased levels of cortisol and glucagon occurred at 30 min, and growth hormone at 40 min after insulin.

Thus, insulin-induced hypoglycemia in man results from both a decrease in glucose production and an increase in glucose utilization. Accelerated glycogenolysis produced much of the initial, posthypoglycemic increment in glucose production. The contribution of glycogenolysis decreased with time, while that of gluconeogenesis from alanine increased. Of the hormones studied, only the increments in plasma catecholamines preceded or coincided with the measured increase in glucose production after hypoglycemia. It therefore seems probable that adrenergic mechanisms play a major role in the initiation of counter-regulatory responses to insulin-induced hypoglycemia in man.

INTRODUCTION

In man, the circulating levels of a number of hormones increase in response to hypoglycemia induced by intravenous insulin administration. Many of these hormones are thought to antagonize the hypoglycemic action of insulin, by modulating either glucose production or glucose utilization or both. Catecholamines (1–3), glucagon (4–9), and cortisol (10, 11) increase the rate of hepatic gluconeogenesis from three-carbon precursors, such as lactate and alanine. Catecholamines and glucagon also accelerate hepatic glycogenolysis (12, 13). Although growth hormone has not been shown to alter gluconeogenesis or glycogenolysis, it does appear to decrease glucose utilization (14). The relative contribution of each of these hormonal responses to the overall defense against hypoglycemia in the intact organism is unclear.

The effects of insulin-induced hypoglycemia on simultaneous glucose and alanine turnovers and precursor-product interrelationships were determined in normal volunteers. Alanine was studied because it is quantitatively the most important precursor, other than lactate, for hepatic gluconeogenesis (15, 16). Circulating levels of epinephrine, norepinephrine, glucagon, cortisol, growth hormone, and insulin were determined frequently

before and after the induction of hypoglycemia, to characterize precisely the pattern and temporal relationships of the metabolic and hormonal responses to hypoglycemia in normal man.

METHODS

Six adult male volunteers, ranging in age from 22 to 41, were selected for this study. All were within 10% of ideal body weight (Metropolitan Life Insurance Company Tables), averaging 74.5±1.8 kg (mean±SEM). No subject received prescription or nonprescription medication other than aspirin for 3 mo before the study. Routine screening blood chemistries, hemograms, and urinalyses were normal. All subjects were hospitalized for at least 1 day before the day of study.

After a 12-h overnight fast, short plastic catheters were inserted in the antecubital veins bilaterally at 0800. After withdrawal of the initial blood samples, a priming intravenous injection of [2-^{3}H]glucose (0.10 μCi/kg) and [U-^{14}C]alanine (0.10 μCi/kg) (both from New England Nuclear, Boston, Mass.) were administered, followed immediately by a continuous 6-h infusion of both isotopes (0.15 μCi/kg per h). The specific activity of [U-^{14}C]alanine was greater than 135 mCi/mmol and that of [2-^{3}H]glucose was 200–600 mCi/mmol. Both isotopes were diluted in sterile 0.9% sodium chloride and infused from the same syringe with a Harvard constant infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). Infusion rates were calibrated after each study and samples of the infusate in the syringe were retained for subsequent counting.

Each subject was maintained on absolute bed rest throughout the study. Venous blood samples were drawn without tourniquet occlusion at −240, −180, −120, −60, −30, and −10 min and immediately before insulin injection. After the 0-time sample, crystalline insulin (0.15 U/kg) was injected intravenously. Blood samples were then obtained at 5-min intervals for the next 30 min, then at 10-min intervals for the next 30 min and at half-hourly intervals thereafter. Blood samples for determinations of intermediates and specific activities were immediately deproteinized in chilled 3 M perchloric acid and neutralized extracts prepared before their storage at −80°C. Samples of blood for insulin and glucagon assays were collected directly in Trasylol (1,000 U/ml; FBA Pharmaceuticals Inc., New York) and those for catecholamine determinations were collected in reduced glutathione. Iced samples were immediately centrifuged and the supernates stored at −20°C or −80°C until assayed.

Alanine (17) and glucose (18) were determined in triplicate with fluorometric enzymatic techniques. Plasma glucagon (19), growth hormone (20), and insulin (21) were determined by double-antibody radioimmunoassay methods. Plasma cortisol levels were assayed by a competitive protein-binding technique (22). Plasma catecholamines were measured by a single-isotope derivative method based on the enzymatic conversion of the catecholamines to their respective metanephrines (23, 24). Labeled metanephrine and normetanephrine were separated by thin-layer chromatography to permit independent measurement of epinephrine and norepinephrine. This method is sensitive to les than 10 pg/ml of epinephrine or norepinephrine. Interassay coefficients of variation, calculated from aliquots of a plasma pool used for internal standardization of the assays reported below, were 8.5% for epinephrine and 3.5% for norepinephrine. The efficiency of separation of one catecholamine from the

other was greater than 99.5% with standard solutions containing up to 2 × 10^6 pg/ml of epinephrine or norepinephrine.

For determinations of their specific activities in plasma, glucose (25) and alanine (26) were recovered from neutralized perchloric acid extracts of blood by ion exchange chromatography techniques. Glucose was isolated by serial anion-cation exchange chromatography on Dowex 50W-X8 and AGI-X8 (Bio-Rad Laboratories, Richmond, Calif.), with recoveries between 94 and 103%. Alanine was isolated by high-pressure (500 lb/in^2), rapid-resolution liquid chromatography on HPB-80 (Hamilton Co., Reno, Nev.) spherical anion exchange resin. The column was warmed to 55°C and elution was performed with a 0.25 M sodium citrate buffer (pH 3.28). Column effluent fractions were assayed for alanine content and the appropriate fractions were pooled, concentrated under dry N$_2$ gas, and reassayed before counting. Alanine recoveries ranged from 92 to 98%. Radioisotope counting was performed in a refrigerated liquid scintillation spectrometer (Searle Analytic Inc., Des Plaines, Ill.). All samples and standards were counted to at least the 1% significance level (40,000 gross counts); samples for background determinations were counted for 100 min. Tritium efficiency averaged 44% and ^{14}C efficiency was 67%, with 11% spill of ^{14}C counts into the tritium channel with the dual-label program settings.

Calculations of glucose inflow (production) and outflow (utilization) from the circulating glucose pool were performed with the equations of Steele et al. (27, 28) for nonsteady state conditions, as modified by Cowan and Hetenyi (29) and subsequently confirmed by Steele et al. (30). Precursor-product interrelationships for glucose and alanine were determined by a modification of the equations of Kreisberg et al. for glucose-lactate interrelationships (25). The present modifications allow for alanine distribution in total body water (31), and correct for loss of ^{14}C label appearing in glucose, due to the fumarase randomization and subsequent decarboxylation by P-enolpyruvate carboxykinase of four-carbon intermediates in the pathway of gluconeogenesis (32, 33). Statistical evaluation of data was performed with Student's t test.

RESULTS

After the intravenous injection of 0.15 U/kg of crystalline insulin, a rapid fall in plasma glucose levels occurred from a preinjection mean (±SEM) of 89±3 mg/dl to 39±4 mg/dl, observed 25 min after insulin injection (Fig. 1). Subsequently, there was an initial rapid increase to 59±4 mg/dl at 50 min, followed by a slower rate of increase to 72±2 mg/dl at 120 min after insulin. 5 min after intravenous insulin injection, the mean serum insulin level exceeded 1 mU/ml (Fig. 2). In the interval 10–40 min after insulin, a semilogarithmic plot of insulin concentrations showed a linear, time-dependent decrease, yielding a calculated half-life of 8.3 min. At the plasma glucose nadir (25 min after insulin), serum insulin levels were 153±22 μU/ml.

Isotope dilution methodology was used to determine changes in glucose kinetics. As shown in Fig. 3B, the specific activity of the tritium-labeled glucose was constant at least 60 min before the injection of insulin. Constant specific activity of the ^{14}C label in the circulating alanine pool was also achieved at least 1 h before the
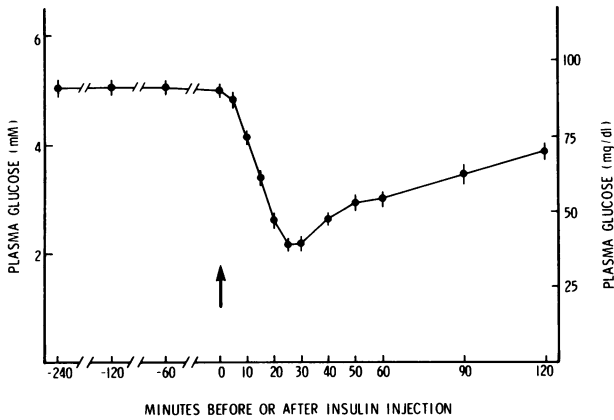
FIGURE 1 Plasma glucose concentrations before and after insulin injection. Glucose was determined enzymatically with hexokinase and glucose-6-phosphate dehydrogenase in neutralized perchloric acid extracts of plasma samples (18), obtained at the indicated times. The arrow drawn at zero time shows the point of insulin injection (0.15 U crystalline insulin/kg body wt). Values plotted are the means±SEM for the six subjects.

administration of insulin (Fig. 4B). The rate of appearance of the ^{14}C label in the circulating glucose pool from labeled alanine was used to determine the rate of gluconeogenesis. As noted in Fig. 3B, nearly constant specific activity of the ^{14}C label in the circulating glucose pool was achieved approximately 60 min before insulin.

At steady state, calculated glucose inflow and outflow were approximately equal at 125 mg/kg per h or 223.4± 12.8 g/day. This rate is in excellent agreement with estimations of total glucose production obtained by others using isotope dilution methods or hepatic vein catheter techniques (34–41). The rate of glucose production from alanine was 17.2±1.1 mg/kg per h, which accounted for 13.6±2.2% of total glucose production. Alanine inflow and outflow before insulin were 448±43 μmol/kg per h. These rates are in excellent agreement with standard calculations of alanine turnover made under steady state assumptions. Approximately one third of the alanine utilization rate was accounted for by glucose production from alanine.

As shown in Fig. 3C, the calculated glucose outflow increased rapidly after insulin injection, to a rate three times basal 15 min after insulin. This increased glucose outflow ceased 25–30 min after insulin and further decreased at 50 min to less than the control rate. On the other hand, glucose production was strikingly inhibited by insulin to 25% of the basal rate 15 min after the injection. However, a marked increase in glucose production was observed to begin in the interval 20–25 min after insulin. Glucose production peaked 50 min after insulin, at a rate 170% of basal.

In contrast to the marked changes in plasma glucose concentrations and glucose kinetics, there was only

a slight decline in plasma alanine levels throughout the study (Fig. 4A). Plasma alanine inflow and outflow rates increased only 20% in the interval 20–25 min after insulin, with a subsequent decrease to control rates 40 min after insulin. A secondary increase in the alanine outflow rate began 1 h after insulin, and this increased for the remainder of the study. Gluconeogenesis from alanine was substantially inhibited atfer insulin injection, decreasing 55% 15 min after insulin injection (Fig. 3A). However, in the interval 20–25 min after insulin, an increase was observed (Fig. 3C). In contrast to the accelerated rate of total glucose production observed 30–60 min after insulin, gluconeogenesis from alanine was not increased over the preinsulin control rates during this interval. From 1 to 2 h after insulin injection, however, a linear increase in gluconeogenesis was observed, which at 2 h was 250% of the preinjection rate.

Plasma cortisol levels (Fig. 5A) remained unchanged from preinjection levels (4.4±0.5 μg/dl) for the first 25 min after insulin, with the first significant increase ($P <$ 0.01) occurring 30 min after insulin, to a concentration (11.5±1.8 μg/dl) approximately 250% of preinsulin values. Maximal cortisol concentrations of 18.2±1.6 μg/dl were observed 60 min after insulin. Plasma glucagon levels (Fig. 5B) were 89±9 pg/ml and remained unchanged for the first 25 min after insulin. Glucagon levels increased significantly ($P < 0.05$) 30 min after insulin injection to a concentration of 157±28 pg/ml, 85% greater than basal ($P < 0.05$). Maximal glucagon levels (181±28 pg/ml) were found 40 min after insulin
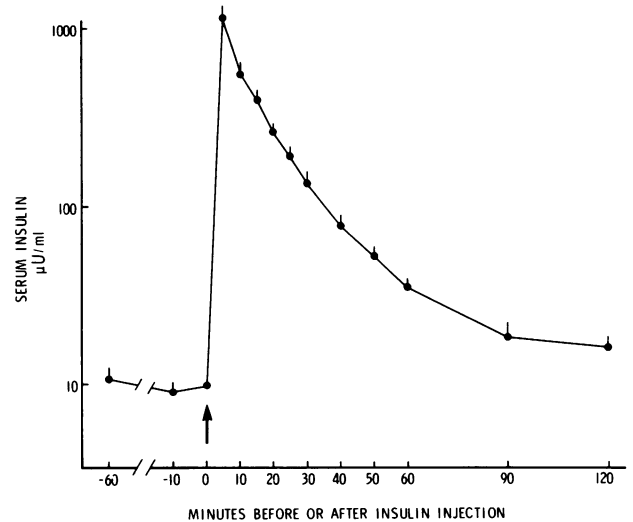

FIGURE 2 Serum insulin concentration before and after exogenous insulin. Insulin was determined by a double-antibody radioimmunoassay method (21). Insulin injection (0.15 U crystalline insulin/kg) is indicated by the arrow at zero time. Values shown are the means±SEM for the six subjects at each time point.
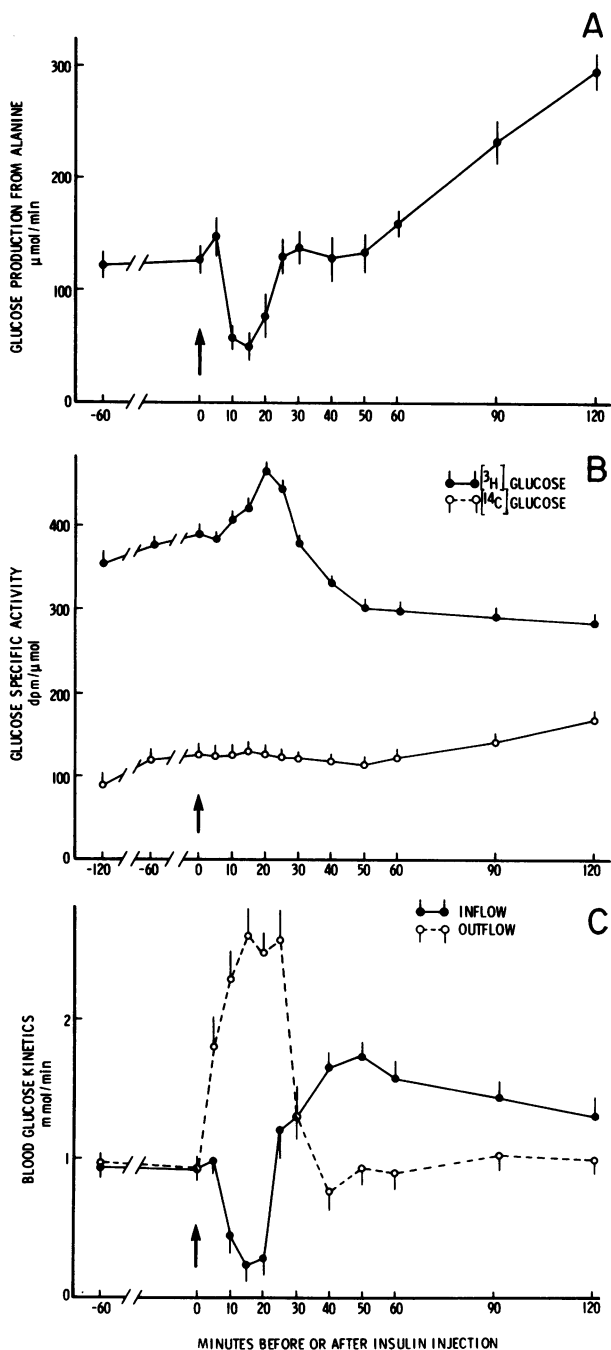
FIGURE 3 Glucose production from alanine (A), tritium, and carbon-14 specific activities in the circulating glucose pool (B), and glucose inflow and outflow before and after insulin injection (C). Glucose was isolated and quantitatively recovered from venous plasma samples with serial anion-cation exchange chromatography, and was assayed enzymatically by a fluorometric procedure with hexokinase and glucose-6-phosphate dehydrogenase. Disintegrations per minute in glucose were calculated after sample counting to at least the 1% significance level. Glucose inflow (production) and outflow (utilization) were calculated by the equations

injection and these decreased linearly thereafter to a concentration only 29% greater than control at 120 min after insulin. Growth hormone levels were low before insulin injection (1.0±0.3 ng/ml), and showed a relatively slow increase after insulin. There was some scatter in the growth hormone responses of individual subjects such that the first significant increase ($P < 0.05$) in growth hormone levels was not observed until 40 min after insulin injection. This increase, however, was more than 20 times basal, and maximal, 40-fold increases in growth hormone were observed at 60 min after insulin. The temporal sequence and magnitude of these increments in cortisol, glucagon, and growth hormone levels induced by hypoglycemia are entirely consistent with previous observations in man (42–45).

The mean plasma epinephrine level (Fig. 6) before insulin was 43±2 pg/ml. A fourfold increase in epinephrine to 166±71 pg/ml ($P < 0.05$) was observed 20 min after insulin, with maximal epinephrine levels (2,200 ±660 pg/ml), 50 times the preinsulin levels, found 50 min after insulin injection. Plasma norepinephrine levels showed qualitatively similar changes. Before insulin, the mean norepinephrine level was 266±36 pg/ml, with the first significant increase ($P < 0.05$) to 457±45 pg/ml observed 25 min after insulin injection. A maximal increase to 770±98 pg/ml was found 50 min after injection.

DISCUSSION

Isotope dilution methodology can be employed to provide a reliable estimation in vivo of whole body flux of metabolites such as glucose (46). The use of [2-^{3}H]glucose allows quantitation of recycled as well as unrecycled glucose production, since glucose-6-phosphate metabolism by phosphohexoseisomerase removes both hydrogen atoms from the C-2 of the glucose molecule (47–51). Mean total glucose production in these normal male subjects was 125.3±7.1 mg/kg per h or 223.4±12.8 g/ day. This value is in good agreement with previously published studies using isotopic or hepatic vein catheter techniques under otherwise similar conditions (34–41). Plasma alanine inflow-outflow calculations yielded rates in good internal agreement with calculations of alanine turnover. Although the fraction of alanine utilization accounted for by gluconeogenesis was approximately one-third of the alanine outflow rate, the amount of glucose derived from alanine was only 13%. The latter value compares favorably with studies using arterio-

of Steele et al. (30). Glucose production from alanine was estimated by a modification of the precursor-product relationships of Kreisberg et al. (25). The time of intravenous insulin injection (0.15 U/kg) is indicated by the arrow at zero time. Values are given as means±SEM for the six subjects and are shown at the end of the time periods to which they correspond.
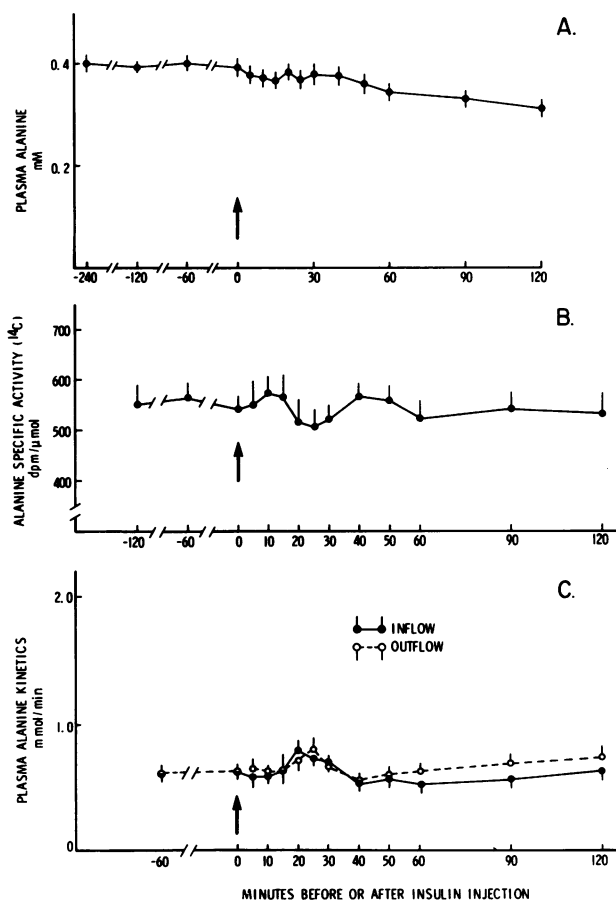
FIGURE 4 Plasma alanine concentration (A), carbon-14 specific activity in alanine (B), and plasma alanine inflow and outflow rates before and after insulin injection (C): Alanine was quantitatively recovered from perchloric acid extracts of venous plasma by high-pressure, high-speed liquid chromatography. Alanine was determined by a semi-microfluorometric, enzymatic assay system using alanine aminotransferase and lactate dehydrogenase (17). Samples were counted in a xylene scintillant to the 1% significance level. The arrow at zero time indicates intravenous insulin injection (0.15 U crystalline insulin/kg). Alanine inflow and outflow were calculated using the equations of Steele et al. (30). Values are given as means±SEM and are shown at the end of this time period to which they correspond.

hepatic venous catheterization procedures in fasting man, showing that alanine utilization accounts for approximately 10% of the observed glucose production (52).

The increased glucose utilization observed in this study with pharmacologic quantities of insulin is entirely consistent with in vitro observations of insulin-stimulated glucose uptake (53–56). Increased glucose utilization was not the only mechanism by which insulin produced the rapid fall in plasma glucose levels. Total glucose production and gluconeogenesis from alanine were

profoundly inhibited by insulin. During the 25 min after insulin, total glucose production decreased from an expected 23.1±1.2 mmol to an observed 14.2±2.2 mmol. In this same interval, glucose outflow increased from an expected 23.8±0.9 to an observed 62.0±5.3 mmol. However, this calculation may well underestimate the contribution of decreased glucose production to the hypoglycemia, since some compensatory increase in glucose production as the plasma glucose level fell would have been anticipated.
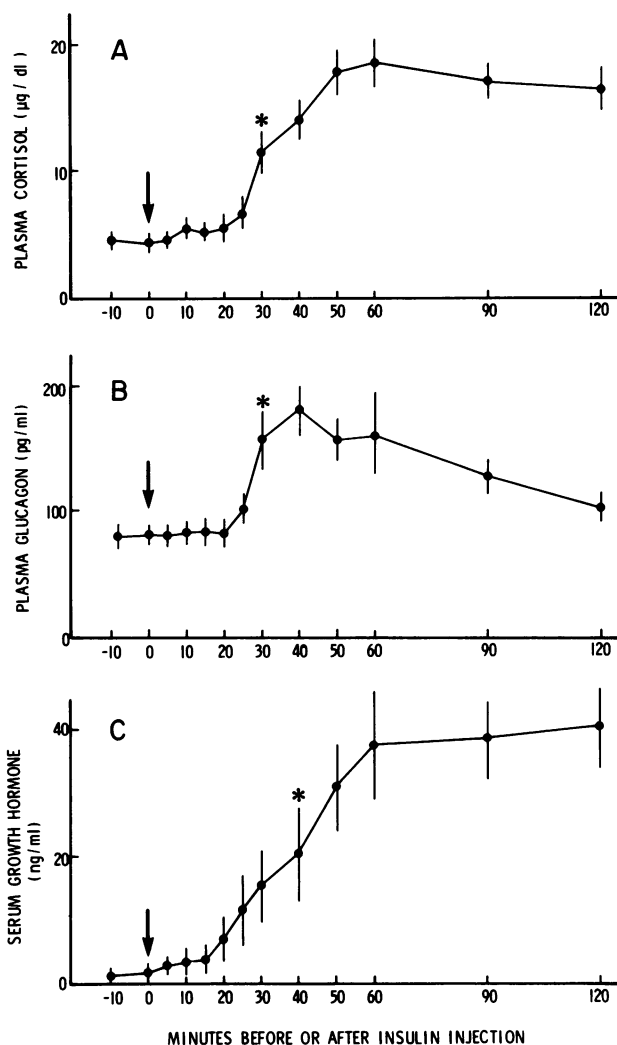

FIGURE 5 Plasma cortisol (A), plasma glucagon (B), and serum growth hormone concentrations (C) before and after insulin injection. Cortisol was determined in venous plasma samples by a competitive protein-binding assay (22), and glucagon and growth hormone were measured with a double-antibody radioimmunoassay (19, 20). The time of insulin injection is shown by the arrow at zero. Values shown are means±SEM for each sample period. Asterisks indicate the first significantly different hormone concentration ($P < 0.05$) as compared to preinjection concentrations.
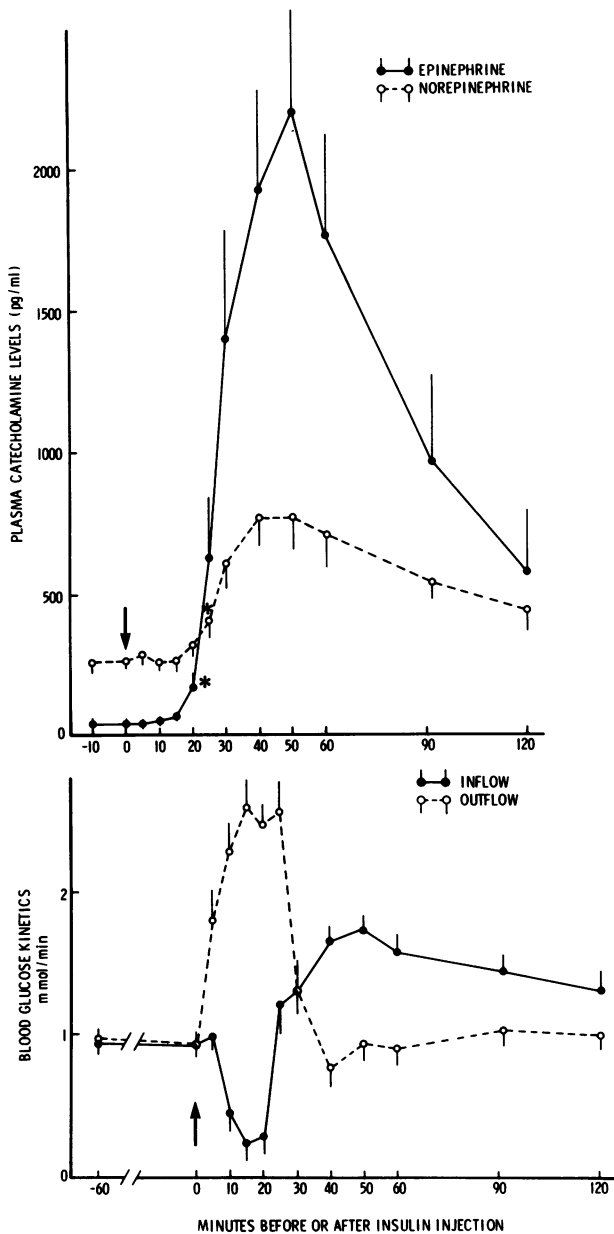
FIGURE 6 Plasma catecholamine concentrations and glucose inflow and outflow before and after insulin injection. Catecholamines were determined by a single-isotope, enzymatic-derivative assay (24) in venous plasma samples collected at the indicated time points. Insulin injection is shown by the arrow at zero time. Values shown are the means±SEM. Glucose inflow-outflow rates from Fig. 3 are shown for temporal comparisons.

In the interval 20–25 min after insulin injection, total glucose production rapidly increased, attaining a maximal rate twice basal at 50 min after insulin. In this same period, gluconeogenesis from alanine returned to basal levels but did not further increase until 60 min after

insulin. The increased gluconeogenesis observed from 1 to 2 h after insulin did not derive entirely from an increased rate of substrate delivery, since the alanine inflow was increased only 15–20% over the control rate. Instead, the increased gluconeogenesis appears to result from a redirection of hepatic alanine metabolism. Before insulin, glucose production from alanine accounted for about one third of total alanine utilization; 2 h after insulin, alanine utilization by gluconeogenesis increased to two thirds of total alanine utilization. Since the initial increase in gluconeogenesis from alanine observed 25–30 min after insulin injection was not greater than the rate observed before insulin, it seems apparent that the twofold increase in total glucose production at 40 min resulted principally from accelerated glycogenolysis.

The increase in glucose production observed 25 min after insulin injection could not have resulted solely from a declining level of insulin, since the absolute insulin concentration at this time was greater than 150 $\mu U/ml$. Instead, metabolic intervention by hormonal or neural factors or both seems evident. Whereas significant increments in plasma glucagon, cortisol, and growth hormone occurred after the activation of glucose production after hypoglycemia, increments in plasma epinephrine clearly preceded and increments in plasma norepinephrine coincided with the activation of glucose production. Epinephrine levels increased nearly fourfold at 20 min and more than 10-fold at 25 min after insulin injection. Although showing qualitatively similar changes, norepinephrine levels increased only 60% 25 min after insulin. However, of the amount of norepinephrine released from sympathetic postganglionic neurons, that which appears in plasma represents only the relatively small fraction that escapes axonal reuptake and local metabolism (57). Thus, it may well be that the magnitude of the norepinephrine discharge during hypoglycemia was comparable to that of epinephrine release. Although this is the first demonstration of an acute increase in plasma norepinephrine (as opposed to epinephrine) during insulin-induced hypoglycemia (58), other investigators using 2-deoxyglucose-induced glucopenia in man have also emphasized the importance of the axonal release of norepinephrine for glucose homeostasis (59, 60). Studies in vivo as well as in vitro have shown that both catecholamines activate glycogenolysis and increase the rate of gluconeogenesis from three-carbon intermediates such as alanine (2, 3, 12, 61, 62). It seems reasonable to conclude, therefore, that increased epinephrine secretion, and perhaps norepinephrine release, may have produced the initial increase in glucose production observed in this study.

An inhibition of insulin-stimulated glucose outflow was a clear concomitant to the hormonal alterations of glucose production in response to hypoglycemia. The increased glucose utilization after insulin fell approxi-

mately 65% in the interval 25–30 min after insulin, with a further decrease to a rate 20% less than basal 50 min after insulin. This decrease cannot be attributed solely to a decrease in circulating insulin levels, because levels of approximately 100 μU/ml were observed during this interval. Of the hormones determined, only a 10-fold increase in circulating epinephrine and a 60% increase in circulating norepinephrine preceded these changes in glucose utilization. Studies in vitro using isolated skeletal muscle preparations have shown that epinephrine addition markedly decreased the rate of glucose uptake by muscle (63–70). It therefore seems possible that the substantially increased levels of catecholamines produced in response to hypoglycemia may have been, at least in part, responsible for the diminution in glucose outflow.

The results of this study indicate that increased epinephrine secretion and norepinephrine release play a major role in the metabolic response to insulin-induced hypoglycemia in man. Direct effects of epinephrine and perhaps of norepinephrine on hepatic glycogenolysis and gluconeogenesis may initiate the increase in glucose production after hypoglycemia. Furthermore, increased catecholamine secretion may also reduce the rate of peripheral glucose utilization, thereby acting to preserve the remainder of the circulating glucose pool. Beyond these direct effects of adrenergic secretion, adrenergic recruitment, and coordination of glucagon, cortisol and growth hormone secretion may serve as an important reinforcement to other mechanisms increasing the secretion of these hormones, thereby augmenting the overall glycemic response. Growth hormone (71–73) and cortisol by way of ACTH secretion (72, 74) are both increased by adrenergic hypothalamic mechanisms. Glucagon secretion can also be increased by adrenergic activity (75–78). These three hormones may well function to augment the early hyperglycemic effects of the catecholamines. Clearly, a role for adrenergic mechanisms in the modulation of more physiologic fluctuations in the plasma glucose concentration remains to be established.

ACKNOWLEDGMENTS

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