Epinephrine Exerts Opposite Effects on Peripheral Glucose Disposal and Glucose-Stimulated Insulin Secretion

A Stable Label Intravenous Glucose Tolerance Test Minimal Model Study

Angelo Avogaro, Gianna Toffolo, Anna Valerio, and Claudio Cobelli

Epinephrine (EPI) plays a pivotal role in regulating glucose metabolism both in splanchnic and peripheral tissues. Nevertheless, previous studies did not clarify the mechanisms by which EPI affect both glucose disposal processes in peripheral tissues and β-cell secretion. The aim of this study was to investigate, in six normal volunteers, the effects of elevated EPI concentration on peripheral glucose disposal and insulin secretion by using the stable labeled (either [6,6-$^2H_2$]or [2-$^2H_1$]glucose) intravenous glucose tolerance test (IVGTT) in conjunction with the minimal models of labeled glucose disappearance and C-peptide secretion. Elevated plasma EPI concentration significantly decreased glucose effectiveness (S_G*) by 29% (0.0059 ± 0.0013 vs. 0.0083 ± 0.0011 min^{-1}, $P < 0.05$), and even more, 61%, insulin sensitivity (S_I*); (22 ± 6 × 10^4 vs. 54 ± 20 × 10^4 $min^{-1} \cdot pmol \cdot l^{-1}$; $P < 0.01$). These findings are not due to an isotopic effect induced by an enhanced glycogen breakdown, because the [2-$^2H_1$]glucose tracer, which is not incorporated into glycogen, gave results similar to those of [6,6-$^2H_2$]glucose tracer. No differences were observed in first phase cell sensitivity, Φ_1, in the EPI study (199 ± 91 vs. 245 ± 144 10^9, NS), but there was a significant increase in the second-phase cell sensitivity to glucose Φ_2, (15.2 ± 1.7 vs. 17.7 ± 4.4 $10^9 \cdot min^{-1}$, $P < 0.05$). In conclusion, EPI selectively impairs peripheral glucose metabolism because of its unique ability to simultaneously and independently decrease glucose effectiveness and insulin sensitivity. Furthermore, EPI enhances Φ2, the ratio between the C-peptide amount secreted during the second phase and the area under the curve of the glucose signal, indicating that the observed increase of C-peptide concentration is due not only to the augmented glucose signal but also to a specific EPI-mediated enhancement of β-cell responsivity to glucose.
***Diabetes* 45:1373–1378, 1996**

From the Department of Clinical and Experimental Medicine and Azienda Ospedaliera di Padova (A.A., A.V.), and the Department of Electronics and Informatics (G.T., C.C.), University of Padova, Padova, Italy.

Address correspondence and reprint requests to Dr. Angelo Avogaro, Cattedra di Malattie del Metabolismo, Via Giustiniani 2, 35100 Padova, Italy.

Received for publication 12 June 1995 and accepted in revised form 10 May 1996.

EPI, epinephrine; IVGTT, intravenous glucose tolerance test; S_G*, glucose effectiveness; S_I*, insulin sensitivity.

Epinephrine (EPI) plays a pivotal role in regulating free fatty acid (1,2), ketone body (3,4), amino acid (5), and glucose metabolism both in splanchnic and peripheral tissues. EPI inhibits splanchnic glucose uptake during an exogenous glucose infusion (6) and enhances hepatic glucose production by stimulating both glycogenolysis and gluconeogenesis (7,8). It has also been shown that, in dogs, the increments of hepatic glucose production during EPI infusion are dose dependent (9).

EPI opposes the biological action of insulin in peripheral tissues as shown several years ago by Deibert and DeFronzo (10). These data have been later strengthened by Rizza et al. (11,12) who also showed that EPI inhibits glucose clearance in man predominantly by β-adrenergic mechanisms. Nevertheless, these studies did not thoroughly clarify the mechanisms by which EPI impairs glucose disposal in peripheral tissues.

Using the hyperglycemic clamp technique, Baron et al. (13) found that EPI inhibits only insulin-mediated but not non-insulin-mediated glucose uptake. However, this experimental approach is not without difficulties, because its interpretation may be confounded by important side effects of somatostatin on glucose metabolism, and by the potential "breakthrough" of insulin secretion in the presence of hyperglycemia (14,15).

For these reasons the intravenous glucose tolerance test (IVGTT) has been viewed as a useful tool to exploit the vast array of metabolic effects that EPI has on glucose metabolism. Using the IVGTT and the minimal model, Martin et al. (16) showed that EPI reduces glucose tolerance, and increases both first- and second-phase sensitivity in dogs. More recently, Morrow et al. (17) have shown that, when plasma EPI concentrations are exogenously increased two- to threefold, there is a substantial decrease of both insulin sensitivity (S_I) and glucose effectiveness (S_G) (17). However, because the unlabeled IVGTT minimal model does not allow separation between hepatic glucose production and glucose disposal, the two parameters S_G and S_I measure not only the effects of glucose and insulin on glucose dis-

posal but also their inhibitory effects on hepatic glucose production (18–20).

Furthermore, it has been shown that EPI, inhibits basal insulin secretion through α2-receptor mechanisms (21,22). However ,the data on EPI effect on glucose-stimulated insulin release are not concordant, because its capability to enhance β-cell secretion has been observed both in vitro (23) and in vivo (24). Moreover, all the above methods only provide a gross picture of the effect of EPI on insulin secretion in vivo.

The aim of this study was thus to investigate the effects of elevated EPI concentration on peripheral glucose disposal and insulin secretion. In particular we measured the two individual determinants of glucose disposal, insulin sensitivity (S_I*) and glucose effectiveness (S_G*), independently of their action on hepatic glucose production by using the stable isotopically labeled IVGTT in conjunction with the minimal model of labeled glucose disappearance. The IVGTT also allows the simultaneous assessment of cell function in the presence of elevated plasma EPI concentrations. In particular, we measured the first- and second-phase β-cell sensitivity to glucose by using minimal model of C-peptide secretion and kinetics (25).

RESEARCH DESIGN AND METHODS

Subjects. A stable isotopically labeled IVGTT was performed on six normal healthy male volunteers who participated to the study after a written informed consent. They were 22 ± 2 years old, and their BMI was 23 ± 1 kg/m^2. The subjects were in good health. For at least 3 days before the study, each subject consumed a diet containing >250 g carbohydrate.

Experimental procedures. On the day of the study, at 7:00 A.M., after an overnight fast, the subjects were admitted to the Divisione di Malattie del Ricambio of the University of Padova. The experimental protocol was approved by the Ethical Committee of the University Hospital of Padova. A 20-gauge butterfly needle was inserted into a dorsal hand vein at 7:30 A.M. The hand was then placed in a box heated to 60°C to arterialize venous blood. The patency of the needle was maintained with a controlled saline infusion throughout the study. An 18-gauge cannula was then placed into the contralateral antecubital vein for injection of the labeled glucose load and EPI infusion. Four subjects were studied twice in a randomized order. On one occasion, starting at 8:00 A.M, they received a continuous infusion of EPI at a rate of 8.2 mmol/min (1.5 μg/min) lasting until 300 min (end of sampling). On another occasion, four subjects were restudied during a continuous saline infusion instead of EPI. Two subjects participated only in the EPI study.

EPI was dissolved in saline solution in the presence of ascorbic acid (0.5 mg/ml) to prevent oxidation. New EPI solutions were prepared every 2 h throughout the test. Ninety minutes after the beginning of EPI infusion, each subject received an IVGTT labeled with the 6,6-$^2H_2$ isotopolog of glucose. However, to exclude possible glycogen cycling of deuterium in position 6 during prolonged EPI infusion, one subject received the 2-$^2H_1$ isotopologue of glucose, which loses its deuterium in the isomerization of glucose-6-phosphate to fructose-6-phosphate. The glucose bolus was administered over 30–60 s. The final concentration of deuterated glucose in the tracer solution was ~10% of the natural unlabeled glucose content. Each subject received a test dose of 300 mg/kg.

Blood samples were obtained before and during the EPI infusion before the IVGTT. After the IVGTT pulse, samples were obtained at –30, –15, 0, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 60, 80, 100, 120, 140, 180, 210, 240, and 300 min. The samples for glucose, deuterated glucose, insulin, and C-peptide determinations were mixed with two drops of heparin, whereas those for EPI determinations with reduced glutathione and EDTA. They were immediately centrifuged at 4°C, and stored at –80°C until the assay.

Materials. [6,6-$^2H_2$]glucose was purchased from MassTrace, Inc. (Woburn, Ma.). [2-$^2H_1$]glucose was purchased from C/D/N isotopes, Inc. Croydon, U.K. Chemical purity was verified by specific enzymatic analysis with glucose oxidase. Sterility was verified by bacteriologic analysis, and the material was shown to be pyrogen-free. Before each study, an appropriate amount of the labeled powder was dissolved in a sterile 50% glucose solution and then passed through a 0.22 μ Millipore filter into a sterile vial that was sealed until use.

Biochemical and stable isotope tracer analysis. Plasma was separated and plasma glucose content determined enzymatically with a glucose analyzer (Beckman, Fullerton, CA). In the remaining plasma, insulin and C-peptide were assayed with specific radioimmunoassay (26) and catecholamines with an HPLC method (27).

Deuterated glucose was analyzed as a pentaacetate derivative using a method previously described (19). The samples were analyzed on a Hewlett-Packard 5988 Quadrupole gas chromatography mass spectrometry instrument operated in the electron impact mode by selected ion monitoring after isothermal separation at 250°C on a 30-m J & W capillary column. Glucose pentaacetate isotopomers are monitored at m/z 242 and 244 for [6,6-$^2H_2$]glucose, and at m/z 242 and 243 for [2-$^2H_1$]glucose as described previously (28).

From ion intensity ratios, the value in the sample of the isotope ratio R, between labeled and unlabeled species was derived. For the [6,6-$^2H_2$]glucose tracer, [6,6-$^2H_2$]glucose and [6,6-$^1H_2$]glucose are the labeled and unlabeled species with, respectively, two ^2H atoms or two ^1H atoms in position 6. Species are thus defined with reference to specific atoms in specific positions (29), and in deriving R we correct analytically for interferences in the mass spectrum from the natural isotopic composition of the other atoms of the monitored ion.

The ratio Z between tracer and tracee mass (or concentrations) in the sample can be evaluated from isotope ratio measurements (29) as

$$Z(t) = \frac{G^*(t)}{G(t)} = \frac{R(t) - R_N}{R_I - R(t)} \frac{I + R_I}{I + R_N} \quad (1)$$

where G^* is the tracer glucose concentration, that is the concentration in the sample of the exogenous administered mixture of natural and deuterated glucose; G is the tracee glucose concentration, that is the concentration of endogenous natural glucose; R_I and R_N are isotope ratios in a sample of pure tracer and tracee respectively.

The above approach based on the Z variable only requires the isotopic indistinguishability assumption. In the dual isotope study (19), a different approach was used, Rationale and assumptions of the two approaches are detailed in (30,31).

Assessment of glucose disposal by the minimal model of glucose disappearance. The minimal model of tracer glucose disappearance has been applied on tracer glucose data to measure parameters reflecting disposal processes only (19,20). The time course of tracer concentration G^* was derived from Z and from total (tracer + tracee) glucose concentration measurement, G_{tot}. Since

$$G_{tot}(t) = G^*(t) + G(t) = G^*(t)\,[1 + 1/Z(t)] \quad (2)$$

one has

$$G^*(t) = G_{tot}(t) \cdot Z(t)/[1 + Z(t)] \quad (3)$$

The minimal model of tracer glucose disappearance is described as

$$G^*(t) = -\,[S_G^* + X(t)]G^*(t) \qquad G^*(0) = G^*_0 \quad (4)$$

$$X(t) = -\,p^*_2\,\{X(t) + S_I^*\,[I(t) - I_b]\} \qquad X(0) = 0 \quad (5)$$

where I is plasma insulin concentration with I_b its basal value and X is a variable related to insulin action. Parameters S_I* (min · pmol^{-1} · l^{-1}) and S_G* (min^{-1}) measure respectively insulin sensitivity and glucose effectiveness, i.e., the effect of glucose per se on its own disposal at basal insulin.

Assessment of insulin secretion by the minimal model of C-peptide secretion. The minimal model of C-peptide secretion (25) has been applied to C-peptide data. The models are

$$\dot{C}P_1(t) = -\,(k_{01} + k_{21})\,CP_1(t) + k_{12}\,CP_2(t) + mx(t) \qquad CP_1(0) = 0 \quad (6)$$

$$\dot{C}P_2(t) = k_{21}\,CP_1(t) - k_{12}\,CP_2(t) \qquad CP_2(0) = 0 \quad (7)$$

$$\dot{x}(t) = -m\,x\,(t) + Y(t) \qquad X(0) = X_0 \quad (8)$$

$$\dot{Y}(t) = -\,\alpha[Y(t) - \beta(G - h)] \qquad Y(0) = 0 \quad (9)$$

Equations 6 and 7 describe C-peptide kinetics, with CP_1 and CP_2 denoting C-peptide concentrations above basal in compartment 1 and 2, respectively. C-peptide secretion, mx, enters compartment 1, and it is a linear function of the amount x of C-peptide in the β-cells. Y represents the provision of new C-peptide to the cells, stimulated by glucose above the threshold h through parameter β. The initial condition x_0 represents the stored C-peptide, and it

TABLE 1
Glucose, insulin, and C-peptide plasma concentrations in the basal and EPI-stimulated periods

	Basal (t = –90')			Pre-IVGTT (t = 0')			End-IVGTT (t = 300')		
	Glucose (mmol/l)	Insulin (pmol/l)	C-Peptide (pmol/l)	Glucose (mmol/l)	Insulin (pmol/l)	C-Peptide (pmol/l)	Glucose (mmol/l)	Insulin (pmol/l)	C-Peptide (pmol/l)
Control study (n = 4)	—	—	—	4.5 ± 0.3	70 ± 28	496 ± 99	4.3 ± 0.1	63 ± 35	430 ± 99
EPI study									
n = 4†	4.4 ± 0.5	70 ± 28	496 ± 99	5.7 ± 0.8*	105 ± 42	595 ± 198	5.2 ± 0.6*	63 ± 21	496 ± 99
n = 6‡	4.4 ± 0.4	70 ± 21	529 ± 132	5.6 ± 0.7*	91 ± 35	595 ± 165	5.3 ± 0.6*	63 ± 14	463 ± 99

*Statistically significant vs. t = –90'. †Mean ± SE in the four subjects who were part of both the control and the EPI studies. ‡Mean ± SE in the six subjects who were part of the EPI study.

is responsible of first phase C-peptide secretion. When divided by the maximum change in plasma glucose ΔG, it measures the first-phase β-cell sensitivity, $F_1 = X_0/\Delta G$. The sensitivity to glucose of the second-phase insulin secretion is given by $\Phi_2 = \beta(\text{min}^{-1})$.

Parameter estimation. A nonlinear least squares estimation technique has been applied to the experimental data of each individual to derive estimates of the unknown parameters of the two models, together with a measure of their precision (32). Measurement errors have been assumed independent, Gaussian, zero mean with experimentally determined coefficient of variation in the range 2–8% for tracer glucose concentration and equal to 6% for C-peptide concentration.

As concerns the model of C-peptide secretion and kinetics, because its identification requires knowledge of C-peptide kinetics, parameters k_{01}, k_{21}, k_{12} have been fixed to standard values in both control and EPI studies (33). An indirect support to the assumption that EPI does not influence C-peptide kinetics is provided by the fact that the 90-min EPI infusion does not alter significantly C-peptide levels (Table 1). The simplified version of the C-peptide model described in appendix B of (25) has been adopted in two control studies.

The statistical significance of differences has been calculated using two-tailed unpaired Student's t test or two-way analysis of variance for repeated measures. A P value <0.05 was considered statistically significant. Values are reported as means ± SD.

RESULTS

Glucose, insulin, and C-peptide: basal values and effect of EPI infusion. Baseline plasma EPI was 539 ± 64 pmol/l. Baseline plasma norepinephrine was 950 ± 56 pmol/ml. Exogenous EPI infusion increased plasma EPI concentration to 1,977 ± 103 pmol/ml. No significant changes were observed in norepinephrine concentration (1,080 ± 62 pmol/l). The coefficient of variation of plasma EPI concentration was 5 ± 4% during the IVGTT time course in the control study, and 13 ± 5% during the EPI study.

As shown in Table 1, baseline plasma glucose, insulin, and C-peptide were all within the normal ranges. EPI infusion significantly increased plasma glucose concentrations from 4.4 ± 0.5 to 5.7 ± 0.8 mmol/l (P < 0.05) at time 0' (beginning of IVGTT). No significant changes were observed in plasma glucose levels between time 0' and time 300' (end of IVGTT) (5.2 ± 0.6 mmol/l).

No significant changes were induced by elevated plasma EPI concentration in either plasma insulin or C-peptide baseline concentrations. The difference in I_b between time 0' mark or (105 ± 42 pmol/l) and time 300' (63 ± 21) was not statistically different. The differences in the two values were determined by the data of one subject (subject 1) who had different values between time 0' and time 300'. This subject also had the highest increase in plasma glucose during EPI infusion.

Glucose, insulin, C-peptide, and tracer glucose concentration during IVGTT. As shown in Fig. 1, during the stable label IVGTT, plasma glucose and tracer glucose were significantly higher in the presence of elevated plasma EPI concentration. A similar pattern was observed for the two different tracers.

Insulin and C-peptide curves were also higher during EPI infusion from time 30 following the glucose bolus until 120–180 min.

Glucose disposal and insulin secretion indexes. The ability of the minimal models of glucose disappearance and C-peptide secretion to fit, respectively, tracer glucose data and C-peptide data is shown in Fig. 1, where the mean of the individual model fits is shown against the data.

The effects of EPI on minimal model indices, S_G*, S_I*, Φ_1, Φ_2 are shown in Table 2. Elevated plasma EPI concentration significantly decreased glucose effectiveness by 29%, and even more, by 61%, insulin sensitivity, S_I*, (Fig. 2).

No differences were observed in first-phase β-cell sensitivity, Φ_1 in the EPI study compared with the control study (199 ± 91 vs. 245 ± 144 10^9, NS). On the contrary, in the four subjects who had both the control and the EPI study, EPI tended to increase Φ2, the second phase cell sensitivity to glucose (14.3 ± 1.3 vs. 12.8 ± 0.7 $10^{-9} \cdot \text{min}^{-1}$, P = NS), and this effect became highly significant when all subjects were included in the statistical analysis (15.2 ± 1.7 vs. 12.8 ± 0.7 $10^{-9} \cdot \text{min}^{-1}$, P < 0.05, two-tailed unpaired t test).

DISCUSSION

Clamp (13) and unlabeled IVGTT-based studies (16,17) have shown that EPI counteracts insulin action on glucose disposal. However, these approaches were not able to dissect the relative role of insulin to inhibit hepatic glucose production and to stimulate glucose disposal in peripheral insulin-sensitive tissues. The stable label IVGTT provides robust information on the role of EPI in counteracting insulin action on peripheral tissues. Our results show that EPI impairs S_I*: because this parameter reflects the effect of insulin on glucose disposal only, we can speculate that EPI-induced reduction in S_I* is to be ascribed to the negative effect of this hormone on glucose utilization processes at the peripheral tissue level. This observation extends the data gathered by Alford and colleagues (34), who showed that EPI affects insulin sensitivity but further supports the concept that skeletal muscle is a major site of EPI-mediated insulin resistance (10,35). In this perspective, a large body of evidence has shown that EPI may inhibit insulin-induced translocation of glucose transporters (36), and it is also able to inhibit insulin-mediated glycogenesis in human skeletal muscle because of an increase in G-6-phosphate, an inhibitor of hexokinase (37,38).

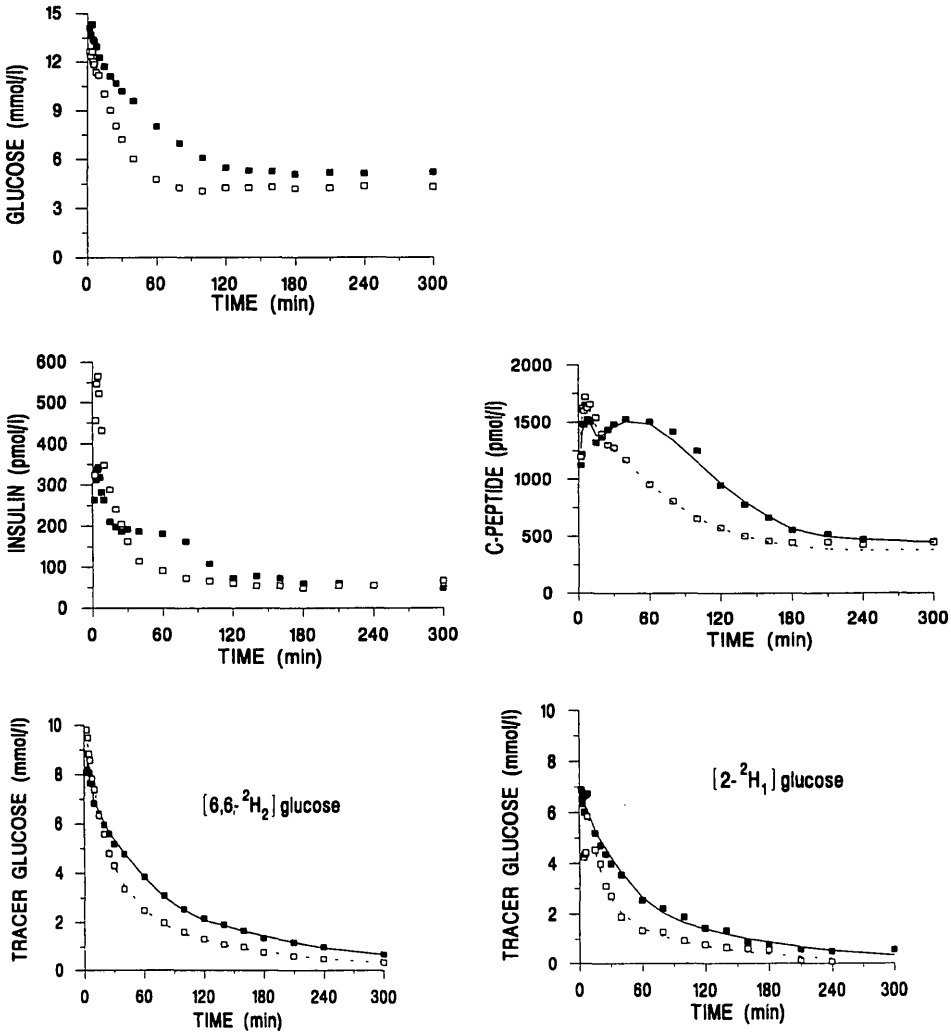

FIG. 1. Average concentration in plasma glucose, insulin, C-peptide, and tracer glucose during IVGTT, in the control study (□) and during EPI infusion (■). The means of the individual fits of the minimal models of glucose disappearance and C-peptide secretion and kinetics are also shown (continuous and dashed line for control and EPI study, respectively) against the data. For the [2-$^2H_1$]glucose tracer, data, and model fit in subject #4 is shown.

Glucose influences its own disposal by a mass action effect (39). Thus it is particularly important to understand the effects of EPI on this process also because non-insulin-mediated glucose disposal accounts for about 70% of basal glucose uptake (13). The stable label IVGTT enabled us to assess this process without the need of a hyperglycemic glucose clamp and the use of somatostatin. We showed that elevated EPI concentrations reduce S_G*, a parameter

TABLE 2
Glucose disposal and insulin secretion parameters

	S_G* (min^{-1})	S_I* (10^4 × min · pmol/l^{-1} · l^{-1})	Φ_1 (10^9)	Φ_2 (10^9 × min^{-1})
Control study				
Subject 1	0.0076 (3)†	61 (5)	118 (7)	13.2 (7)
Subject 2	0.0083 (3)	25 (3)	302 (10)	12.6 (17)
Subject 3	0.0074 (3)	69 (4)	138 (5)	13.6 (6)
Subject 4§	0.0099 (4)	62 (5)	422 (20)	11.9 (3)
Mean ± SD	0.0083 ± 0.0011	54 ± 20	245 ± 144	12.8 ± 0.7
Epinephrine study				
Subject 1	0.0059 (6)	18 (6)	76 (12)	16.0 (5)
Subject 2	0.0047 (5)	16 (5)	302 (3)	13.8 (7)
Subject 3	0.0064 (4)	31 (7)	141 (5)	12.8 (5)
Subject 4	0.0078 (5)	18 (8)	161 (9)	14.6 (5)
Subject 5	0.0065 (4)	21 (9)	213 (7)	16.6 (4)
Subject 6	0.0041 (6)	29 (6)	302 (6)	17.3 (6)
Mean ± SD (n = 4)‖	0.0062 ± 0.0013‡	21 ± 7‡	170 ± 95	14.3 ± 1.3
Mean ± SD (n = 6)¶	0.0059 ± 0.0013‡	22 ± 6‡	199 ± 91	15.2 ± 1.7‡

†Precision of parameter estimate expressed as percent CV. ‡Statistically significant (P < 0.05) from control study. §Received [2-$^2H_1$]glucose labeled IVGTT. ‖Subjects 5 and 6 who do not have the control study, not included. ¶Subjects 5 and 6 included.

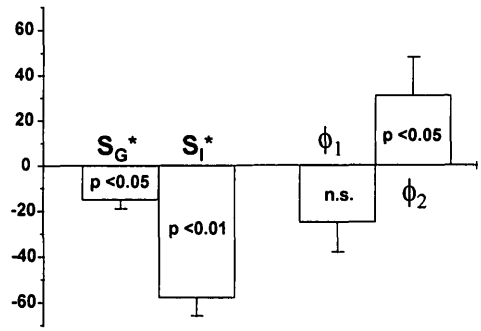

FIG. 2. Percentage changes during EPI study of glucose disposal and insulin secretion parameters.

that measures the stimulatory effect of glucose per se on its own peripheral disposal at basal insulin, by almost 30%. By using the unlabeled IVGTT, Morrow et al. (17) also showed a decrease in the S_G parameter in the elderly even in the presence of small elevations of plasma EPI concentrations, whereas this effect was not observed in insulin-dependent diabetic patients (34). Again, the parameter S_G also measures the effect of glucose in inhibiting its own production at basal insulin, and thus it is not possible to know the relative role of EPI in affecting glucose effectiveness in the liver and in peripheral tissue. Our results are not directly comparable with those of Baron et al. (13) and Laakso et al. (40) who showed that EPI is able to affect only insulin mediated glucose uptake. In fact, the above studies measured non-insulin-mediated glucose uptake using the hyperglycemic glucose clamp technique, which requires a severe state of insulin deficiency whereas S_G* measures glucose effectiveness at basal insulin. Moreover, in those studies, the non-insulin-mediated glucose uptake in muscle during EPI infusion was predicted only indirectly by extrapolating whole body and leg glucose uptake at 0 insulin. The observation that EPI inhibits the glucose uptake at basal insulin may lead to the hypothesis that some non-insulin-depedent intracellular pathways of glucose disposal are altered by this hormone. The accumulation of glucose-6-phosphate itself, and the ability of EPI to counteract glucose transport by inhibiting the intrinsic activity of GLUT4 (41), may explain the reduction in glucose effectiveness. The fact that, in the present study, EPI decreased the glucose utilization at basal insulin by 30% raises the question in which tissue this parameter of glucose disposal could be potentially affected by this hormone. The central nervous system accounts for almost 70% of non-insulin-mediated glucose uptake, and the non-nervous tissues account for the remaining 30%, 13% being due to muscle uptake. Although, we must bear in mind that the transport rate of glucose in the brain is at least one order of magnitude greater than phosphorylation (42), it is likely that the EPI-induced inhibition of glucose uptake might play a role at the brain level. Glucose transport into the brain of diabetic animals appears to be depressed as a consequence of hyperglycemia (43,44). Therefore EPI, by increasing plasma glucose concentrations, might have an indirect role in decreasing brain glucose uptake.

A possible source of error in the estimate of insulin sensitivity and glucose effectiveness during EPI infusion might be due to the site of the label within the glucose moiety. EPI is a powerful stimulator of glycogenolysis. Because we used [6,6-$^2H_2$]glucose as tracer, which could be incorporated into glycogen by the direct pathway and recycled back into plasma glucose via glycogenolysis, higher isotope ratio of this tracer might have been observed. However, also with [2-$^2H_1$]glucose, which loses its label immediately in the phosphohexose isomerase reaction and thus is not incorporated into glycogen, a difference was observed in the presence of elevated EPI concentrations. This observation definitively excludes, at least in one subject, an isotopic effect induced by an enhanced glycogen breakdown due to elevated EPI concentrations.

It has been shown that EPI inhibits basal insulin secretion (21) and insulin gene transcription (45). However, its acute effects after a glucose challenge are not concordant. We have shown that the first phase was slightly depressed during the EPI-stimulated study: this observation was consistent with that of Morrow et al. (17) who showed that the acute insulin response to the IVGTT bolus was somewhat depressed in the elderly at EPI levels similar to those we had in our subjects. However, because they calculated the acute insulin response as the mean rise in plasma insulin concentration at 3, 4, and 5 min after the intravenous infusion, they were able to give only a gross assessment of first-phase insulin secretion only. Our study is the first report on the effects of EPI on the dynamics of insulin secretion. We have shown that elevated EPI concentrations slightly decrease the total amount of C-peptide secreted during the first phase in response to the glucose load and reduces, albeit not significantly, Φ1, i.e., the first-phase β-cell sensitivity to glucose. On the other hand, elevated EPI concentrations enhance Φ2, the second-phase β-cell sensitivity to glucose. Because Φ2 is the ratio between the C-peptide amount secreted during the second phase and the area under the curve of the glucose signal, an increased Φ2 during EPI infusion means that the observed increase of C-peptide concentration is due not only to the augmented glucose signal but also to a specific EPI-mediated enhancement of β-cell responsivity to glucose.

Our data are consistent with both in vitro (23) and in vivo (24) findings, which show that infusion of EPI at low concentration produces a potentiation of insulin secretion in response to glucose. Why this happens is a matter of speculation: one possible explanation is that because EPI increases the intracellular concentration of glucose-6-P, a major trigger for insulin release, EPI may enhance β-cell secretion independently from the prevailing plasma glucose concentration (46).

In conclusion, the stable labeled IVGTT provides evidences that EPI selectively impairs peripheral glucose metabolism because of its unique ability to simultaneously and independently decrease glucose effectiveness and insulin sensitivity. Furthermore, EPI induces a significant increase of second-phase pancreatic sensitivity due to a specific EPI-mediated enhancement of β-cell responsivity to glucose.

ACKNOWLEDGMENTS

This study was supported by a Consiglio Nazionale delle Ricerche (CNR) grant N. and a Ministero dell'Università, Ricerca Scientifica e Tecnologica (MURST) grant N.

91.00446.PF40, and National Institutes of Health Grant RR-02176.

REFERENCES

1. Fain JN, Garcia-Sainz JA: Adrenergic regulation of adipocytes metabolism. *J Lipid Res* 24:945–966, 1983
2. Arner P, Griegholm E, Engfeldt P, Bolinder J: Adrenergic regulation of lipolysis in situ at rest and during exercise. *J Clin Invest* 85:893–898, 1990
3. Avogaro A, Cryer PE, Bier DM: Epinephrine's ketogenic effect in humans is mediated principally by lipolysis. *Am J Physiol* 263:E250–E260, 1992
4. Avogaro A, Valerio A, Gnudi L, Maran A, Miola M, Duner E, Marescotti MC, Iori E, Tiengo A, Nosadini R: The effects of different plasma insulin concentrations on lipolytic and ketogenic responses to epinephrine in normal and type I (insulin-dependent) diabetic humans. *Diabetologia* 35:129–138, 1992
5. Del Prato S, De Fronzo RA, Castellino P, Wahren J, Alvestrand A: Regulation of amino acid metabolism by epinephrine. *Am J Physiol* 258:E878–887, 1990
6. Saccà L, Vigorito C, Cicala M, Ungaro B, Sherwin RS: Mechanisms of epinephrine-induced glucose intolerance in normal humans. Role of the splanchnic bed. *J Clin Invest* 69:284–293, 1981
7. Saccà L, Vigorito C, Cicala M, Corso G, Sherwin RS: Role of gluconeogenesis in epinephrine-stimulated glucose production in humans. *Am J Physiol* 245:E294–E302, 1983
8. Waldhausl WK, Gasic S, Bratusch-Marrain P, Komjati M, Korn A: Effect of stress hormones on splanchnic substrates and insulin disposal after glucose ingestion in healthy humans. *Diabetes* 36:127–135, 1987
9. Stevenson RW, Steiner KE, Connoly CC, Fuchs H, Alberti KGMM, Williams PE, Cherrington AD: Dose-related effects of epinephrine on glucose production in conscious dogs. *Am J Physiol* 260:E363–E370, 1991
10. Deibert DC, DeFronzo RA: Epinephrine-induced insulin resistance in man. *J Clin Invest* 65:717–721, 1980
11. Rizza RA, Haymond MW, Cryer PE, Gerich JE: Differential effects of epinephrine on glucose production and disposal in man. *Am J Physiol* 237:E356–E362, 1979
12. Rizza RA, Cryer PE, Haymond MW, Gerich JE: Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. *J Clin Invest* 682–689, 1979
13. Baron AD, Wallace P, Olefsky JM: In vivo regulation of non-insulin-mediated and insulin-mediated glucose uptake by epinephrine. *J Clin Endocrinol Metab* 64:889–895, 1987
14. Caruso M, Divertie GD, Jensen MD, Miles JM: Lack of effect of hyperglycemia on lipolysis in humans. *Am J Physiol* 259:E542–E547, 1990
15. Avogaro A, Gnudi L, Valerio A, Maran A, Miola M, Opportuno A, Tiengo A, Bier D: Effects of different plasma glucose concentrations on lipolytic and ketogenic responsiveness to epinephrine in type I (insulin-dependent) diabetic subjects. *J Clin Endocrinol Metab* 76:845–850, 1993
16. Martin IK, Weber KM, Boston RC, Alford FP, Best JD: Effects of epinephrine infusion on determinants of intravenous glucose tolerance in dogs. *Am J Physiol* 255:E668–E673, 1988
17. Morrow LA, Morganroth GS, Herman WH, Bergman RN, Halter JB: Effects of epinephrine on insulin secretion and action in humans. Interaction with aging. *Diabetes* 42:307–315, 1993
18. Cobelli C, Pacini G, Toffolo G, Saccà L: Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT. *Am J Physiol* 250:E591–E598, 1986
19. Avogaro A, Bristow JD, Bier DM, Cobelli C, Toffolo G: The stable-label IVGTT: insulin sensitivity and glucose effectiveness from minimal models of (2-^2H_1) and ($6,6\text{-}^2H_2$)glucose kinetics. *Diabetes* 38:845–853,1989
20. Caumo A, Giacca A, Morgese M, Pozza G, Micossi P, Cobelli C: Minimal models of glucose disappearance: lessons from the labeled IVGTT. *Diabetic Med* 8:1–11, 1991
21. Robertson RP, Porte D Jr.: Adrenergic modulation of basal insulin secretion in man. *Diabetes* 22:1–8, 1973
22. Porte D Jr: A receptor mechanism for the inhibition of insulin release by epinephrine in man. *J Clin Invest* 46:86–94, 1967
23. Lacey RJ, Cable HC, James RF, London NJ, Scarpello JH, Morgan NG: Concentration-dependent effects of adrenaline on the profile of insulin secretion from isolated human islets of Langherans. *J Endocrinol* 138:555–563, 1993
24. Ostenson CG, Hjemdahl P, Efendic S: Release of catecholamines is increased but does not contribute to the impaired insulin secretion in the perfused pancreata of diabetic rats. *Pancreas* 8:34–38, 1993.
25. Toffolo G, De Grandi F, Cobelli C: Estimation of beta-cell sensitivity from IVGTT. C-peptide data knowledge of the kinetics avoids errors in modeling the secretion. *Diabetes* 44:845–854, 1995
26. Kuzuya H, Blix PM, Horowitz DL, Steiner DF, Rubenstein AH: Determinations of free and total insulin and C-peptide in insulin treated diabetics. *Diabetes* 26:22–29, 1977
27. Hyemdahl P: Catecholamine measurements by high performance liquid chromatography. *Am J Physiol* 247:E13–E20, 1984
28. Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA, Kipnis DM: Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes* 26:1016–1023, 1977
29. Cobelli C, Toffolo G, Foster D: Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am J Physiol* 262:E968–975, 1992
30. Avogaro A, Vicini P, Valerio A, Caumo A, Cobelli C: The hot but not the cold minimal model allows precise assessment of insulin sensitivity in NIDDM subjects. *Am J Physiol* 270:E532–E540, 1996
31. Cobelli C, Vicini P, Toffolo G, Caumo A: The hot IVGTT minimal models: simultaneous assessment of disposal indices and hepatic glucose release. In *Proceedings MINMOD '94*. Lovejoy J, Bergman R, Eds. Pennington Nutrition Series, Baton Rouge, Louisiana State University Press, in press
32. Carson ER, Cobelli C, Fikelstein L: *The Mathematical Modeling of Metabolic and Endocrine Systems*. New York, Wiley, 1983
33. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels. *Diabetes* 41:368–377, 1992
34. Walters JM, Ward GM, Kalfas A, Best JD, Alford FP: The effect of epinephrine on glucose-mediated and insulin-mediated glucose disposal in insulin-dependent diabetes. *Metab Clin Exp* 41:671–677, 1992
35. Capaldo B, Napoli R, Di Marino L, Saccà L: Epinephrine directly antagonizes insulin-mediated activation of glucose uptake and inhibition of free fatty acid release in forearm tissues. *Metab Clin Exp* 41:1146–1149, 1992
36. Smith U, Kuroda M, Simpson IA: Counter-regulation of insulin-stimulated glucose transport by catecholamines in the isolated rat adipose cell. *J Biol Chem* 259:8758–8763, 1984
37. Chiasson JL, Shikama H, Chu DTW, Exton J: Inhibitory effect of epinephrine on insulin stimulated glucose uptake by rat skeletal muscle. *J Clin Invest* 68:706–713, 1981
38. Raz I, Katz A, Spencer MK: Epinephrine inhibits insulin-mediated glycogenesis but enhances glycolysis in human skeletal muscle. *Am J Physiol* 260:E430–E435, 1991
39. Capaldo B, Santoro D, Riccardi G, Perrotti N, Saccà L: Direct evidence for a stimulatory effect of hyperglycemia per se on peripheral glucose disposal in type II diabetes. *J Clin Invest* 77:1285–1290, 1986
40. Laakso M, Edelman SV, Brechtel G, Baron AD: Effects of epinephrine on insulin-mediated glucose uptake in whole body and leg muscle in humans: role of blood flow. *Am J Physiol* 263:E199–204, 1992
41. Bonen A, Megeney LA, McCarthy SC, McDermott JC, Tan MH: Epinephrine administration stimulates GLUT4 translocation but reduces glucose transport in muscle. *Biochem Biophys Res Commun* 187:685–691, 1992
42. Lund-Andersen H: Transport of glucose from blood to brain. *Physiol Rev* 59:305–352, 1979
43. McCall J: The impact of diabetes on the CNS. *Diabetes* 41:557–570, 1992
44. Gyedde A, Crone C: Blood-brain glucose transfer: repression in chronic hyperglycemia. *Science* 214:456–457, 1981
45. Redmon JB, Towle HC, Robertson RP: Regulation of human insulin gene transcription by glucose, epinephrine, and somatostatin. *Diabetes* 43:546–551, 1994
46. Malaisse WJ, Sener A, Herchuelz A, Hutton JC: Insulin release: the fuel hypothesis. *Metab Clin Exp* 28:373–386, 1979