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Insulin Stimulates Epinephrine Release Under Euglycemic Conditions in Humans

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In healthy subjects, acute physiological hyperinsulinemia induces activation of the sympathetic nervous system, but in the absence of hypoglycemia, plasma epinephrine levels have not been found to increase during insulin administration. However, the venous level of epinephrine reflects the net result of release, clearance, and uptake and therefore is not a good measure of adrenomedullary epinephrine secretion. The influence of 90 minutes of euglycemic physiological hyperinsulinemia (60 $mU \cdot m^{-2} \cdot min^{-1}$; plasma insulin concentration, $\approx$700 $pmol \cdot L^{-1}$) on epinephrine kinetics using the ^{3}H-epinephrine tracer method was studied in 12 healthy normotensive, non-obese subjects. After bolus injection, [^{3}H]-epinephrine was continuously infused with arterial and venous blood sampling at regular intervals, enabling calculation of total body (systemic) and forearm epinephrine release and clearance. Studies were performed in the basal state and during sympathetic stimulation by lower-body negative pressure (LBNP) of −15 mm Hg for 15 minutes. Control experiments ("sham" clamps, but with LBNP) were performed in four of the 12 individuals. Euglycemic hyperinsulinemia (all arterial glucose samples $\geq$ 4.2 $mmol \cdot L^{-1}$) induced an increase of the arterial epinephrine concentration ($P = .03$), and tended to increase total body epinephrine release ($P = .08$). Total body epinephrine clearance did not change during hyperinsulinemia. The insulin-induced increase in forearm blood flow ([FBF] by plethysmography, from 3.0 ± 0.4 to 3.8 ± 0.6 $mL \cdot dL^{-1} \cdot min^{-1}$, $P = .01$) was strongly correlated with the increase in arterial epinephrine ($r = .78$, $P < .01$). Plasma epinephrine concentrations did not change during control experiments (sham clamp). Sympathetic stimulation alone as induced by LBNP did not stimulate epinephrine release. However, the combination of insulin and LBNP significantly increased epinephrine release (from 0.37 ± 0.06 to 0.56 ± 0.12 $nmol \cdot m^{-2} \cdot min^{-1}$, $P = .03$). We conclude that acute physiological hyperinsulinemia under euglycemic conditions induces epinephrine release. This effect is enhanced when hyperinsulinemia is combined with sympathetic stimulation by LBNP. Due to increased forearm removal, venous epinephrine concentrations hardly change. Epinephrine release was strongly correlated with the hemodynamic effects of insulin.

BESIDES ITS KEY ROLE in the regulation of metabolism,[1] it has become clear that insulin also has important cardiovascular effects in humans. Acute hyperinsulinemia induces activation of the sympathetic nervous system as measured by plasma norepinephrine concentration, direct measurement of muscle sympathetic nerve activity traffic (microneurography),[2,3] and norepinephrine kinetic studies.[4,5] The increased sympathetic noradrenergic activity to skeletal muscle inducing vasoconstriction is normally overridden by a vasodilator action of insulin.[2,6] In contrast to the effects of insulin on norepinephrine release, the relationship between insulin and adrenomedullary function has received little attention. Although plasma norepinephrine concentrations increase during acute insulin administration,[2,7,8] plasma epinephrine levels are generally reported not to change under euglycemic conditions.[2]

The plasma epinephrine concentration is a function of both the rate of epinephrine secretion into the plasma from the adrenal medullae and the rate of clearance of epinephrine from the circulation.[9] The interpretation of the venous plasma epinephrine concentration as an index of adrenomedullary activity is therefore flawed by the rapid removal of epinephrine from the circulation by many tissues. For example, there is a ±50% extraction of epinephrine across the forearm,[10] which will especially affect venous plasma epinephrine levels. Thus,

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the measurement of venous plasma epinephrine levels during physiological and pathophysiological states may not be an adequate reflection of adrenomedullary function in humans. Moreover, because venous plasma epinephrine concentrations in healthy individuals are low, a very sensitive laboratory method is needed to measure the small increments in plasma epinephrine reliably.[11]

If insulin would increase the adrenomedullary secretion of epinephrine, it may be of clinical relevance, because it would increase systolic and decrease diastolic blood pressure, as well as increase the (forearm) blood flow and heart rate,[12] all factors that cause hyperdynamic circulation.[13] As such, the effects of epinephrine resemble those found during acute hyperinsulinemia,[2] and an insulin-induced increase in the epinephrine concentration may therefore explain part of the reported relationship between hyperinsulinemia/insulin resistance and hyperkinetic circulation.[13,14] In addition, increased circulating epinephrine levels during hyperinsulinemia may contribute to the increase in norepinephrine levels, because it has been shown that epinephrine facilitates neuronal release of norepinephrine by presynaptic stimulation of β-adrenoceptors.[15]

The aim of the present study was to quantify the response of epinephrine kinetics to hyperinsulinemia in detail using the ^{3}H-epinephrine tracer technique.[10,11,16]

SUBJECTS AND METHODS

Subjects

The study group consisted of 12 healthy volunteers. All met the inclusion criteria: age between 25 and 60 years, nonsmoker, absence of hypertension (office blood pressure < 160/90 mm Hg, measured after 5 minutes' rest in the supine position), and body mass index (BMI) less than 27 kg · m^{-2}. Participants used no medication. They were selected by advertisement and received payment. All subjects had normal glucose tolerance and a negative family history of diabetes and hypertension. All participants provided written informed consent. The experimental protocol was approved by the hospital ethics committee.

Protocol and Procedures

During the experiments, subjects remained in the supine position in a quiet, temperature-controlled room (25° to 26°C). Experiments were performed after an overnight fast. A 20-gauge catheter (Angiocath; Becton Dickinson, Sandy, UT) was inserted into the left brachial artery (after local anesthesia with lidocaine HCl) and connected with an arterial pressure monitoring line to a Hewlett Packard (Atlanta, GA) 78353B monitor. The arterial line was kept patent with 0.9% NaCl/heparin infusion (2 U heparin/mL NaCl, 3 mL/h). In the ipsilateral arm, a catheter (20 gauge, 32 mm) was inserted into a deep forearm vein to obtain venous blood samples. On the contralateral side, an identical catheter was inserted into a large forearm vein for infusion of ^{3}H-epinephrine, insulin, and 20% glucose in water.

At least 30 minutes of rest were included after complete instrumentation. Then, a priming dose of 15 μCi · m^{-2} ^{3}H-epinephrine was administered, followed by continuous infusion of 0.35 μCi · m^{-2} · min^{-1} during the entire experiment. Again, 30 minutes were required to obtain a steady state, after which baseline hemodynamic and endocrine measurements were performed. Subsequently, lower-body negative pressure (LBNP) at −15 mm Hg using a perspex box was applied for 15 minutes to stimulate the sympathetic nervous system.[17] Thereafter, 30 minutes of rest were allowed to permit all parameters to return toward the baseline level.

After this second baseline, a hyperinsulinemic-euglycemic clamp was started in which insulin (Actrapid; Novo-Nordisk, Copenhagen, Denmark) 430 pmol · m^{-2} · min^{-1} (60 mU · m^{-2} · min^{-1}) was infused during 90 minutes. The arterial plasma glucose was clamped at the fasting level by a variable glucose infusion rate adjusted by measuring arterial plasma glucose levels every 5 minutes.[18]

Finally, with the continuation of insulin and glucose infusion, a second LBNP at −15 mm Hg was applied for 15 minutes.

Forearm blood flow (FBF) was measured using mercury-in-silastic strain-gauge, venous occlusion plethysmography as previously described.[19] The collecting cuff around the upper arm was inflated to a pressure of 40 mm Hg during eight heart cycles using a Hokanson E20 rapid cuff inflator. The strain gauges were connected with the Hokanson EC4 plethysmograph (Hokanson, Bellevue, WA).

Time Control Experiments

In four male subjects randomly selected from the study group, the entire experiment was repeated on a separate day but without administration of insulin (and glucose) and tritiated epinephrine. Arterial and venous lines were placed as in the original protocol, and LBNP was applied similarly, twice for 15 minutes. During the 90-minute sham clamp period, arterial blood was sampled every 5 minutes, and subjects received a variable saline infusion in an amount identical to the infusion rate of 20% glucose during the original experiment. Blood samples for determination of epinephrine were taken at the same time intervals and processed identically.

Analytical Methods

The plasma glucose level was measured in duplicate, in arterial blood samples that were immediately centrifuged for 20 seconds, by the glucose oxidation method (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Plasma insulin levels were measured with a double-antibody radioimmunoassay (interassay coefficient of variation [CV], 6.2%). Plasma C-peptide was measured with a commercially available double-antibody radioimmunoassay (Diagnostic Products, Los Angeles, CA; interassay CV, 4.3%). Hemoglobin A_{1c} (HbA_{1c}) was measured using a high-performance liquid chromatographic technique (Bio-Rad Laboratories, Veenendaal, The Netherlands; reference value, 4.8% to 6.2%).

Tritiated epinephrine (levo-[*N*-methyl-^{3}H]epinephrine; specific activity, 55 to 85 Ci · mmol^{-1}) was obtained from Du Pont, New England Nuclear ('s Hertogenbosch, The Netherlands). It was sterilized using a micropore filter (0.22 μm) and diluted in 0.9% NaCl containing glacial acetic (0.2 mol/L) and ascorbic acid (1 mg · mL^{-1}). Sterilization, dilution, and batch division were performed under nitrogen. Aliquots of about 70 μCi · mL^{-1} ^{3}H-epinephrine were stored at −80°C until the morning of the experiment, which was always within 3 months from preparation. The weight of the syringe containing the radiotracer was measured just before and after the infusion to verify the infusion rate. At the end of the experiment, an aliquot of the radiotracer was frozen and stored at −80°C for later analysis.

Blood samples for measurement of plasma catecholamine levels were collected in prechilled tubes on melting ice, containing glutathione (0.2 mol/L) and EGTA (0.25 mol/L), and centrifuged at 4°C. Plasma was stored at −20°C; analyses of plasma samples and infusate occurred within 2 months from collection. Plasma samples were analyzed for concentrations of unlabeled and tritium-labeled epinephrine by high-performance liquid chromatography with fluorometric detection after precolumn derivatization with the selective detection agent 1,2-diphenylethylenediamine. The laboratory procedure is a modification of a previously published method, and has been extensively validated in our laboratory.[11] The detection limit of unlabeled epinephrine was 3.2 pmol · L^{-1}, and the intraassay CVs were 3.4% at a plasma concentration of 0.11 nmol · L^{-1} and 7.2% at 0.15 nmol · L^{-1}. The detection limit of ^{3}H-epinephrine was 6 dpm; the interassay CV for ^{3}H-epinephrine was 7% in venous plasma samples.

Calculations and Data Analysis

Kinetic variables were calculated according to the formulas,

$$\text{Total body clearance (TCL, L} \cdot \text{m}^{-2} \cdot \text{min}^{-1}) = [\text{infusion rate (dpm} \cdot \text{m}^{-2} \cdot \text{min}^{-1})]/{}^{3}\text{H-E (dpm} \cdot \text{L}^{-1}),$$

$$\text{Total body E spillover (TSO, nmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}) = \text{TCL} \times [\text{E}]_{art}\ (\text{nmol} \cdot \text{L}^{-1}),$$

$$\text{Forearm E spillover (FSO, pmol} \cdot \text{dL FAV}^{-1} \cdot \text{min}^{-1}) = \text{FPF} \times ([\text{E}]_{ven} - [\text{E}]_{art} + \text{FE} \times [\text{E}]_{art}), \text{ and}$$

$$\text{Forearm removal (FRM, pmol} \cdot \text{dL FAV}^{-1} \cdot \text{min}^{-1}) = \text{PFP} \times \text{FE} \times [\text{E}]_{art},$$

in which

$$\text{Forearm plasma flow (PFP, mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}) = (1 - \text{Hematocrit}) \times \text{FBF},$$

FAV = forearm volume (measured by volume plethysmography), and

$$\text{Fractional extraction (FE)} = ({}^{3}\text{H-E}_{art} - {}^{3}\text{H-E}_{ven})/{}^{3}\text{H-E}_{art}.$$

Forearm vascular resistance (FVR) was calculated by dividing the mean arterial pressure and FBF and expressed in arbitrary units (AU). Pulse pressure was calculated by subtracting diastolic from systolic blood pressure.

Data on norepinephrine kinetics from this study group were used as controls in a report on non–insulin-dependent diabetes mellitus (NIDDM).[5]

During the last 30 minutes of the clamp, the glucose infusion rate was calculated as micromoles per kilogram per minute to obtain whole-body glucose uptake, a measure of insulin sensitivity.[20]

The effects of insulin on epinephrine kinetic and hemodynamic variables were assessed using repeated-measures ANOVA (Student *t* test for paired measurements). In addition, multiple regression analysis was used to explain changes in epinephrine kinetic variables, using previous changes as covariates. To explain the eventual changes of the epinephrine variables due to the combined effect of insulin infusion and LBNP, and to investigate the possibility that the effects of insulin infusion and LBNP might interact, regression models with interaction terms were applied. Multiple correlation coefficients (R^2) were calculated to determine the goodness-of-fit of the models and to estimate the proportion of explained variance. Correlations between variables were calculated using Spearman's rank-correlation tests. Statistical analyses were performed using a SAS (Cary, NC) computer software package. Results in the text, tables, and figures are expressed as the mean ± SEM unless otherwise indicated. Statistical significance was set at P less than .05.

RESULTS

Baseline Measurements

The 12 subjects (eight males and four females) were aged 46.2 ± 6.0 years (mean ± SD; range, 36 to 59) and had a BMI of 24.3 ± 1.7 kg · m^{-2}. They were normotensive (systolic blood pressure, 126 ± 10 mm Hg; diastolic blood pressure, 79 ± 7 mm Hg) and had normal fasting glucose (5.1 ± 0.4 mmol · L^{-1}) and HbA_{1c} levels (5.5% ± 0.5%). Fasting insulin was 56 ± 14 pmol · L^{-1}, and fasting plasma C-peptide was 0.40 ± 0.07 nmol · L^{-1}. Baseline epinephrine kinetic parameters (arterial and venous plasma concentration, total body and forearm spillover, and total body and forearm clearance) are denoted in Table 1.

Characteristics of the subjects in whom control experiments were performed resembled those of the whole study group (age, 44.0 ± 2.6 years; BMI, 25.2 ± 1.6 kg · m^{-2}).

Metabolic Response to Euglycemic-Hyperinsulinemic Clamp

Blood glucose values during the clamp procedure were stable in all individuals (last 30 minutes: mean glucose concentration, 4.9 ± 0.1 mmol · L^{-1}; CV, 4.3% ± 0.5%). Plasma insulin was 651 ± 40 pmol · L^{-1} after 60 minutes and 645 ± 48 pmol · L^{-1} after 90 minutes of insulin infusion. Whole-body glucose uptake, as an index of insulin sensitivity and calculated from the last 30 minutes infusion rates, was 48.9 ± 2.0 (range, 34.5 to 58.4) μmol · kg^{-1} · min^{-1}. During all clamp procedures, arterial plasma glucose was never less than 4.2 mmol · L^{-1}.

Epinephrine Kinetics

Absolute epinephrine kinetic data for the entire experiment and the results of univariate statistical analyses are listed in Table 1.

Table 1. Epinephrine Kinetic Data in the Experimental and Control Groups (mean ± SE)

Parameter	Baseline 1	LBNP	Baseline 2	Insulin/Vehicle‖	Insulin + LBNP
Arterial E (nmol · L^{-1})					
Experimental	0.25 ± 0.03	0.27 ± 0.04	0.24 ± 0.04	0.34 ± 0.05†	0.58 ± 0.10§
Control	0.29 ± 0.07	0.28 ± 0.05	0.26 ± 0.04	0.28 ± 0.06	0.34 ± 0.05
Venous E (nmol · L^{-1})					
Experimental	0.09 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.13 ± 0.02†	0.17 ± 0.03
Control	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.04	0.06 ± 0.01	0.06 ± 0.01
TSO (nmol · m^{-2} · min^{-1})	0.28 ± 0.04	0.27 ± 0.05	0.28 ± 0.06	0.37 ± 0.06‡	0.56 ± 0.12§
TCL (nmol · m^{-2} · min^{-1})	1.09 ± 0.05	0.97 ± 0.06*	1.09 ± 0.06	1.07 ± 0.05	0.95 ± 0.07§
TSO (pmol · dL^{-1} · min^{-1})	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	−0.02 ± 0.02	−0.12 ± 0.07
FRM (pmol · dL^{-1} · min^{-1})	0.31 ± 0.05	0.27 ± 0.06	0.28 ± 0.04	0.50 ± 0.12†	0.68 ± 0.22

NOTE. Data refer to the experimental group unless otherwise indicated.

Abbreviations: E, epinephrine; TSO, total body E spillover, TCL, total body E clearance; FSO, forearm E spillover; FRM, forewarm E removal.

*$P < .05$ *v* baseline 1.

†$P < .05$ *v* baseline 2.

‡$P = .08$ *v* baseline 2.

§$P < .05$ *v* insulin.

‖In control experiments normal saline was infused.

LBNP. During LBNP alone, arterial, venous, and total body epinephrine spillover remained unchanged, but calculated total body epinephrine clearance decreased significantly. After this first LBNP, all parameters returned to baseline values. LBNP in the control experiment induced no changes in arterial and venous epinephrine concentrations (Table 1).

Insulin. After 90 minutes of insulin infusion, arterial and venous epinephrine concentrations were increased ($P = .03$ and .009, respectively). The percentage increase in arterial epinephrine was 53% ± 22% ($P = .031$), and in venous epinephrine 64% ± 24% ($P = .023$). During insulin, total body epinephrine spillover tended to increase, but this did not reach statistical significance ($P = .08$). The percentage increase was 51% ± 20% ($P = .027$). Total body epinephrine clearance did not change during insulin infusion ($P = .67$). Forearm epinephrine removal increased ($P = .04$), but (as expected[11]) forearm epinephrine spillover did not change.

Regression analysis revealed that the changes in arterial epinephrine, venous epinephrine, and total epinephrine spillover during insulin infusion could not be explained by previous changes due to LBNP (proportion of explained variance due to the effects of previous LBNP ≤ 0.05 for each dependent variable).

During the sham clamps, arterial and venous epinephrine concentrations did not change.

Insulin + LBNP. In contrast to the response to LBNP alone, during insulin + LBNP, both arterial epinephrine and total body epinephrine spillover increased significantly (Table 1). Venous epinephrine showed a tendency to increase ($P = .09$). Baseline levels at the start of this LBNP were higher due to the previous insulin effect. When the increments to LBNP on both occasions were calculated as the absolute or relative increase, the response to insulin + LBNP was clearly increased as compared with LBNP alone (percent increase in arterial epinephrine 7.7% ± 7.8% during LBNP alone *v* 76% ± 21% during LBNP + insulin, $P = .019$, percent increase in total body epinephrine spillover −3.0% ± 9.9% during LBNP alone *v* 50% ± 14% during LBNP + insulin, $P = .02$).

During insulin + LBNP, total body epinephrine clearance decreased significantly ($P = .02$). This change was similar to the decrease observed with LBNP alone ($P = .04$) (percentage decrease in total body epinephrine clearance during LBNP alone 11% ± 5% *v* LBNP + hyperinsulinemia 12% ± 4%, $P = .81$). Also, the observed changes in the venous epinephrine concentration and forearm epinephrine removal were similar during insulin + LBNP and LBNP alone.

Multiple Regression Analysis

The variance of changes in total body epinephrine spillover in response to insulin + LBNP is largely explained by the previous changes of total body epinephrine spillover by insulin infusion ($P = .001$) and the interaction between the effects of insulin infusion and LBNP ($P = .003$). The eventual effect of the previous LBNP alone does not contribute to the explanation ($P = .30$). The proportion of explained variance (R^2) of changes of total body epinephrine spillover during insulin + LBNP by the regression model including the interaction term is .83. Without the interaction term, the proportion of explained variance of the model is .43 (Table 2).

Table 2 also shows that the changes in total body epinephrine clearance due to insulin + LBNP can largely be explained by the previous effects of insulin infusion, LBNP alone, and the effect of the interaction ($R^2 = .64$). Without the interaction term, the proportion of explained variance of the model is only .09. This finding indicates that interaction effects of changes due to insulin infusion and LBNP should be taken into account to explain clearance effects of insulin + LBNP.

In the control experiments, the second LBNP induced a slight increase in arterial epinephrine concentrations that was clearly less pronounced than during hyperinsulinemia. The venous epinephrine concentration did not change during the second LBNP in the control experiments.

Hemodynamic Changes

LBNP. LBNP induced vasoconstriction but did not change the blood pressure (Table 3) or heart rate in either the experimental group or the control.

Insulin. Ninety minutes of hyperinsulinemia induced forearm vasodilation. The absolute increase in the arterial epinephrine concentration during insulin was highly correlated with the increase in FBF ($r = .78$, $P < .01$; Fig 1), as was the percent increase in arterial epinephrine (correlation with ΔFBF, $r = .72$, $P = .008$; with ΔFVR, $r = -.76$, $P = .004$).

Systolic blood pressure did not change while diastolic blood pressure decreased during insulin. As a result, pulse pressure increased significantly (from 59 ± 2 to 63 ± 2 mm Hg, $P < .001$). The heart rate did not change. In the control group, sham clamping did not affect FBF or blood pressure (Table 3).

Insulin + LBNP. Similar to LBNP alone, LBNP in combination with hyperinsulinemia induced vasoconstriction but did not change blood pressure or heart rate. Again, the control group

Table 2. Results of Multivariate Analysis

			LBNP		Insulin infusion		LBNP × Insulin		Explained Variance
Parameter	β_0	*P*	β_1	*P*	β_2	*P*	β_2	*P*	(R^2)
Arterial E	0.20	.01	−1.20	.21	0.28	.46	33.8	.004	.74
Venous E	0.01	.55	−0.78	.31	0.36	.29	29.2	.06	.65
TSO	0.04	.37	−0.57	.30	1.13	.001	15.0	.003	.83
TCL	−0.02	.63	0.49	.04	1.1	.02	5.4	.008	.64
FRM	−0.03	.86	−0.84	.68	0.77	.06	1.9	.63	.49

NOTE. Regression analysis of changes of epinephrine kinetic variables in response to insulin + LBNP with previous changes in response to LBNP alone and insulin infusion as covariates, including interaction. Model for analyzing change of each dependent variable: insulin + LBNP = $\beta_0 + \beta_1 \times \text{LBNP}_{\text{alone}} + \beta_2 \times \text{insulin} + \beta_3 \times \text{LBNP}_{\text{alone}} \times \text{insulin}$. Estimation of parameters and proportion of explained variance (R^2).

Abbreviations: E, epinephrine; TSO, total E spillover; TCL, total E clearance; FRM, forearm E removal.

Table 3. Hemodynamic Changes in the Experimental and Control Groups (mean ± SE)

Parameter	Baseline 1	LBNP	Baseline 2	Insulin/Vehicle	Insulin ± LBNP
FBF (mL · dL^{-1} · min^{-1})					
Experimental	3.0 ± 0.4	2.5 ± 0.5*	3.0 ± 0.4	3.8 ± 0.6†	2.6 ± 0.4†
Control	2.3 ± 0.2	1.6 ± 0.3*	1.9 ± 0.2	1.7 ± 0.1	1.5 ± 0.1§
FVR (AU)					
Experimental	34 ± 4	47 ± 7*	35 ± 5	27 ± 3†	46 ± 9‡
Control	40 ± 3	59 ± 10‖	49 ± 5	57 ± 4	62 ± 5
SBP (mm Hg)					
Experimental	125 ± 3	125 ± 4	126 ± 3	128 ± 3	126 ± 3
Control	127 ± 5	123 ± 5	128 ± 5	135 ± 4	130 ± 3
DBP (mm Hg)					
Experimental	66 ± 2	67 ± 2	67 ± 2	65 ± 2	66 ± 2
Control	66 ± 2	66 ± 2	67 ± 2	69 ± 2	68 ± 3

*$P < .05$ *v* baseline 1.
†$P < .05$ *v* baseline 2.
‡$P < .05$ *v* insulin.
§$P = .06$ *v* insulin.
‖$P = .06$ *v* baseline 1.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

showed comparable hemodynamic responses to the second LBNP (Table 3).

DISCUSSION

The major observation of the present study is that short-term physiological hyperinsulinemia induces systemic release of epinephrine, even under strict euglycemic conditions. The increase in plasma epinephrine correlated strongly with the observed increase in skeletal muscle blood flow, suggesting that a relevant part of the vascular and hemodynamic effects of insulin may be mediated by epinephrine.

Sympathetic Stimulant Effects of Insulin

In several but not all[21] studies, it has now been shown that acute insulin administration activates the sympathetic nervous system in healthy humans[2,3] and causes an increase in arterial and venous plasma norepinephrine concentrations,[4,5,8,22] (along with an increase in muscle sympathetic nerve activity).[2,3,8,22] The increase in norepinephrine is due to increased systemic spillover (and hence probably increased neural release), as can be derived from norepinephrine kinetic studies.[4,5] Although the mechanism of insulin-induced sympathetic activation is not well understood, one of the major determinants of sympathetic responses to insulin may be a direct vasodilator response of insulin itself.[2,4-6,8,22]

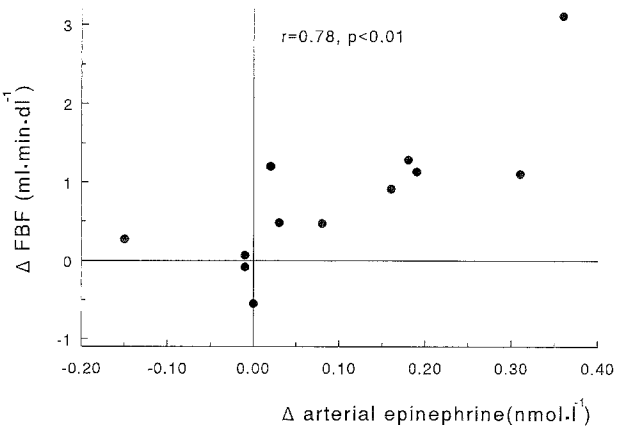

Fig 1. Correlation between absolute change in arterial plasma epinephrine level (x-axis) and absolute change in FBF (y-axis) in response to 90 minutes of hyperinsulinemia.

Effects of Insulin on Adrenomedullary Secretion

The adrenomedullary system is an important part of the autonomic nervous system.[23] Sympathetic activation may also induce epinephrine release from the adrenal medulla. Epinephrine in turn facilitates the release of norepinephrine from sympathetic nerve endings.[15] Despite the activation of the sympathetic nervous system, hyperinsulinemia induces vasodilation.[2] Therefore, the vasoconstrictive effects of norepinephrine may be counterbalanced by a β_2-adrenoceptor–mediated vasodilator effect of epinephrine. Previous studies have indeed suggested that the effects of insulin on the cardiovascular system can be inhibited by the β-adrenoceptor blocker propranolol.[24,25] As such, the literature provides indirect evidence to hypothesize that insulin might induce epinephrine release.

It is generally assumed that plasma epinephrine concentrations do not increase during euglycemia, despite high insulin concentrations. However, looking at previous studies in more detail, several suggest a small, nonsignificant increase. For example, in the study by Anderson et al,[2] venous epinephrine increased from 28 ± 5 to 35 ± 7 pg · mL^{-1} after 60 minutes of euglycemic hyperinsulinemia. In another study by Scherrer et al,[22] venous epinephrine increased during a 1-hour euglycemic-hyperinsulinemic clamp from 0.30 ± 0.05 to 0.36 ± 0.05 nmol · L^{-1}, and in Vollenweider et al,[8] from 0.45 ± 0.07 to 0.52 ± 0.08 nmol · L^{-1}. A recent study even reported a reproducible significant epinephrine increase in NIDDM subjects during clamps.[26] In general, most studies report at least a tendency to increase. Earlier animal studies showed that after insulin injection, plasma epinephrine levels were already increased before hypoglycemia developed.[27]

The results in our study have been obtained with the use of the tritium-labeled epinephrine kinetic technique.[10] Although

venous epinephrine concentrations increased slightly in the present study, the absolute changes were small and could easily have been missed by less sensitive laboratory assays. Most of the studies mentioned measured epinephrine concentrations in venous or arterialized blood, but this is not equal to arterial epinephrine concentrations.[28,29] In addition, arterialization has often been achieved by a hand-warming device, which may be a confounding factor.

Mechanism of Adrenomedullary Activation

While we have found that insulin induces systemic epinephrine release, the study was not designed to explain the mechanism of these effects. Several possible mechanisms have to be considered.

The experimental procedure itself can be regarded as a sympathetic stimulus and might lead to an increase in norepinephrine and/or epinephrine levels. Although one report has suggested this possibility,[30] apart from ours, at least two other studies found no changes during control procedures.[3,31] With the use of epinephrine tracer kinetics, we show that the observed increase in arterial plasma epinephrine is due to increased epinephrine release, since epinephrine clearance did not change. This indicates that under these conditions, the change in the arterial epinephrine concentration reflects only the change in epinephrine release. Because arterial plasma did not change in control experiments, it seems unlikely that the present findings can be explained as a consequence of the experimental procedure itself.

It is well known that hyperinsulinemia in combination with hypoglycemia can stimulate adrenomedullary epinephrine secretion. In our study, hypoglycemia was carefully avoided. Moreover, nearly all subjects showed a small increase in arterial epinephrine concentrations and there were no extreme outliers, which argues against this possible mechanism. Furthermore, it has to be emphasized that in most studies on hypoglycemia, the hyperinsulinemic clamp procedure is used, and therefore, the observed increase in epinephrine concentration may have been the result of both hypoglycemia and hyperinsulinemia. Indeed, several studies have now identified increased epinephrine release in response to hypoglycemia during high insulin concentrations, as compared with low insulin concentrations.[32-34] These findings support our conclusions.

It is unlikely that the first LBNP procedure influenced the response of epinephrine kinetics to insulin. First, the LBNP procedure did not induce an increase in epinephrine secretion. Second, all parameters were back to baseline before the insulin infusion was started (Table 1). Finally, regression analysis showed no evidence of an effect of the first LBNP on the changes induced by insulin infusion.

As already mentioned, insulin causes activation of the sympathetic nervous system, at least in acute experiments. Therefore, insulin infusion can be regarded as a sympathetic stimulus. In general, sympathetic stimuli may induce predominantly norepinephrine or epinephrine release depending on the type of stimulus, a concept known as differentiated sympathetic outflow. Furthermore, sympathetic efferent traffic may vary in different organ systems. Therefore, along this line of evidence and based on our results, it may be possible that hyperinsulinemia, even under euglycemic conditions, induces epinephrine secretion in addition to norepinephrine release. Since hyperinsulinemia did not change epinephrine clearance, the increments in arterial plasma epinephrine levels must be the consequence of an increased adrenomedullary release. This production of epinephrine may occur predominantly in the adrenal medullae, although we cannot exclude extra-adrenal production, as described previously.[35] Activation of the adrenal medulla could be postulated to occur by central activation of preganglionic sympathetic nerve endings.

The increments in arterial plasma epinephrine are small, and may appear unlikely to produce direct significant hemodynamic effects. However, plasma concentrations are not necessarily representative of the effects of epinephrine at the level of the vascular adrenoceptor site. Although it is possible that epinephrine facilitates neuronal norepinephrine release by presynaptic β-adrenergic stimulation, it is not known whether the changes in plasma epinephrine are sufficient for this effect. Others also have described interactions between insulin and the β-adrenergic signal pathway.[36,37]

Effects of Hyperinsulinemia on Adrenergic Responses to Sympathetic Stimuli

The epinephrine response to insulin was enhanced during the sympathetic stimulus of LBNP. This augmented response of epinephrine release was also observed in the control experiments, but to a much lesser extent. One possible explanation may be that the insulin-induced vasodilation in leg muscle causes a more pronounced reduction of venous return in response to LBNP, with a subsequently more intense sympathoadrenal response. Although the two LBNP procedures may therefore differ in intensity, a number of arguments oppose this possibility: (1) The vasoconstrictive responses were similar during both procedures; (2) We have performed a similar study on norepinephrine kinetics in both diabetic patients and healthy subjects, and found no differences in absolute or percentage changes of total body and forearm norepinephrine spillover in response to LBNP alone or in combination with hyperinsulinemia[5]; (3) A more intense stimulus would be expected to result in changes in blood pressure during the LBNP procedure, as a result of deactivation of the arterial baroreceptor (as, for example, during LBNP exceeding −20 mm Hg).[38]

Multiple regression analysis (Table 2) showed that the variance of changes in total body epinephrine spillover in response to insulin + LBNP can be explained in part by the previous response to insulin infusion. The effect of the previous LBNP alone does not contribute to the explanation. The proportion of explained variance is considerably increased (from 0.43 to 0.83) if the interaction of the effects of previous insulin infusion and LBNP is included in the regression model. With respect to total body epinephrine clearance during insulin + LBNP, the analysis shows that interaction effects of insulin infusion and LBNP must be taken into account to explain clearance effects. However, the physiological interpretation of these interaction effects are presently not clearly understood.

The multiple regression analysis of changes in the epinephrine kinetic variables due to the combined effect of insulin infusion and LBNP is a retrospective univariate analysis; it does not take into account the simultaneous distribution of the epinephrine kinetic variables. The analysis cannot overcome the limitations of the design and results of the experiment; in particular, the physiological interpretation of the interaction

between the effects of insulin infusion and LBNP needs further exploration.

In conclusion, we report that in healthy non-obese, normotensive individuals, acute physiological euglycemic hyperinsulinemia induces epinephrine secretion. This effect is amplified during the sympathetic stimulus LBNP. However, due to increased forearm epinephrine removal, venous concentrations hardly change. The epinephrine release could explain part of the hemodynamic effects of insulin. The role of epinephrine in the vascular actions of insulin warrants further research.

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