Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia

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Paramore, Deanna S., Carmine G. Fanelli, Suresh D. Shah, and Philip E. Cryer. Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E872–E881, 1998.—Plasma norepinephrine (NE) concentrations are a fallible index of sympathetic neural activity because circulating NE can be derived from sympathetic nerves, the adrenal medullas, or both and because of regional differences in sympathetic neural activity. We used isotope dilution measurements of systemic and forearm NE spillover rates (SNESO and FNESO, respectively) to study the sympathochromaffin system during prolonged standing, hyperinsulinemic euglycemia, and hyperinsulinemic hypoglycemia in healthy humans. Prolonged standing led to decrements in blood pressure without increments in heart rate, the pattern of incipient vasodepressor syncope. FNESO was not increased (0.58 ± 0.20 to 0.50 ± 0.21 pmol·min⁻¹·100 ml tissue⁻¹), suggesting that the approximately twofold increments in plasma NE and SNESO were derived from sympathetic nerves other than those in the forearm (with a possible contribution from the adrenal medullas). Hyperinsulinemia per se (euglycemia maintained) stimulated sympathetic neural activity, as evidenced by increments in FNESO (0.57 ± 0.11 to 1.25 ± 0.25 pmol·min⁻¹·100 ml tissue⁻¹, P < 0.05), but not adrenomedullary activity. Hypoglycemia per se stimulated adrenomedullary activity (plasma epinephrine from 190 ± 70 to 1720 ± 320, pmol/l, P < 0.01). Although SNESO (P < 0.05) and perhaps plasma NE (P < 0.06) were raised to a greater extent during hyperinsulinemic hypoglycemia than during hyperinsulinemic euglycemia, FNESO was not. Thus these data do not provide direct support for the concept that hypoglycemia per se also stimulates sympathetic neural activity.

sympathetic nervous system; adrenal medullas; epinephrine; hypoglycemia

Although the plasma norepinephrine (NE) concentration is often viewed as an index of sympathetic neural activity, circulating NE can be derived from sympathetic postganglionic nerves, the adrenal medullas, or both (45). Furthermore, the plasma NE concentration is the result of the rate of NE appearance in plasma (conventionally referred to as the NE spillover rate (16) because the vast majority of the NE released from axon terminals of sympathetic postganglionic nerves is dissipated locally by neuronal reuptake or metabolism and does not enter the circulation (45)) and the rate of NE disappearance from plasma (often expressed as the plasma NE metabolic clearance rate). Thus measurements of NE kinetics are required to document the extent to which an increment in the plasma NE concentration is the result of increased spillover (an index of release) or decreased clearance (16). Nonetheless, the systemic plasma NE spillover rate, like the plasma NE concentration, is a marker of sympathetic neural activity, adrenomedullary activity, or both. Furthermore, regional variations in sympathetic neural activity can complicate interpretation of the plasma NE concentration (16).

Approaches to quantitation of sympathetic neural activity per se include 1) direct measurement with microneurography (4, 46, 56), 2) measurement of tissue NE concentrations with microdialysis (24, 31), and 3) NE isotope dilution measurements across a specific organ or tissue that does not include the adrenal medullas (16). Although it is more sensitive than plasma NE concentrations to changes in sympathetic neural activity (22), microneurography is technically demanding, limited to measurement of muscle and skin sympathetic neural activity, and, because it becomes progressively more stressful over time, not optimal for prolonged or repeated experiments. Tissue NE levels are difficult to quantitate with microdialysis (10, 24, 31); not only is a sensitive assay required, but calibration is problematic. Based on these considerations, and given our earlier experience with measurements of systemic NE kinetics (32), we selected measurements of forearm NE kinetics (11, 29) to quantitate sympathetic neural activity. This requires only the addition of deep venous sampling and measurements of forearm blood flow (23) to the basic method (32).

The forearm NE spillover technique also has limitations. First, it provides only an index of sympathetic neural NE release because the vast bulk of NE released is dissipated locally and does not enter the circulation (45). Second, it reflects NE release in only one region and therefore cannot assess regional differences in sympathetic neural activity (34). Third, the tracer technique per se involves several assumptions. For example, the fundamental assumption of the systemic NE clearance method and thus the systemic NE spillover calculation that the tracer mixes with a constant fraction of NE released at sympathetic nerve terminals has been challenged (10, 14). Furthermore, although we have found no differences between systemic NE kinetic data calculated from arterial and arterialized venous sampling but substantially higher values calculated from venous sampling (32), the optimal sampling site has not been determined (40). Nonetheless, as an index of NE release from sympathetic postganglionic
neurons, and thus of sympathetic neural activity, forearm NE spillover rates (FNESO) have been found to be increased during lower body negative pressure (27, 29), during euglycemic hyperinsulinemia (29), during angiotensin II infusion (11), and after a meal (54). We used this technique to distinguish sympathetic neural from adrenomedullary activation during prolonged standing, hyperinsulinemic euglycemia, and hyperinsulinemic hypoglycemia in healthy human subjects.

**METHODS**

Subjects. Five healthy subjects (3 women and 2 men) gave their written consent to participate in study 1. Their mean ± SD age was 30 ± 5 yr (range 24–36 yr), and their mean body mass index (BMI) was 24.2 ± 7.6 kg/m² (range 16.6–36.6 kg/m²). Five healthy subjects (3 women and 2 men) gave their written consent to participate in study 2. Their mean ± SD age was 35 ± 4 yr (range 28–40 yr), and their mean BMI was 23.6 ± 1.5 kg/m² (range 22.2–25.5 kg/m²). Both studies were approved by the Washington University Radioactive Drug Research Committee and the Washington University Human Studies Committee and were performed in the outpatient facilities of the Washington University General Clinical Research Center (GCRC).

Protocols. Subjects presented to the GCRC early in the morning after an overnight fast. A catheter was inserted in a retrograde fashion in a deep antecubital vein in the dominant arm; this was flushed frequently with saline. In the contralateral arm, intravenous lines were inserted into a hand vein, with that hand kept in an ~65°C box to provide arterialized venous samples and in an antecubital vein for infusions ([3H]NE in both studies and, in study 2, insulin and glucose). Equipment for venous occlusion plethysmography (venous occlusion cuff, mercury strain gauge, and wrist occlusion cuff) was placed on the arm with the deep antecubital intravenous catheter.

In study 1, subjects were studied on two occasions in random sequence, separated by at least 1 mo, one time in the supine position throughout [supine in the first 30-min time segment (T1) and supine in the second 30-min time segment (T2)], the supine to supine limb, and one time in the supine position in T1 and after 30–60 min in the standing position in T2 (60 min in the first two subjects, who found this difficult to complete, and 30 min in the next three subjects), the supine to prolonged standing limb. In study 2 subjects were studied, in the supine position, on three occasions in random sequence each separated by at least 1 mo, one time during saline infusion in T1, T2, and the third time segment (T3), the control limb, one time during saline infusion in T1 and insulin infusion with maintenance of euglycemia (see below in T2 and T3), the euglycemic limb, and one time during saline infusion in T1, insulin infusion with euglycemia in T2, and insulin infusion with hypoglycemia (see below in T3, the euglycemic to hypoglycemic limb. Arterialized venous and deep antecubital venous samples (obtained simultaneously) for NE mass and radioactivity, forearm blood flow measurements, arterialized venous samples for hormone and metabolic substrate/intermediate levels, assessments of symptoms, and heart rate and blood pressure measurements were obtained serially, and the electrocardiogram was monitored throughout during hypoglycemia.

NE kinetics were calculated from arterialized venous samples obtained 20, 25, and 30 min into 30-min infusions of [3H]NE (levo-[ring-2,5,6-3H]NE, 40–60 Ci/mmol; New England Nuclear, Boston, MA; 10 nCi·kg⁻¹·min⁻¹) as described previously (32). [3H]NE concentrations and NE specific activities (NE SA) were determined after organic extraction of NE from plasma (32). The systemic NE metabolic clearance rate (SNEMCR) and spillover rate (SNESO) were calculated as

\[
\text{SNEMCR (l/min)} = \frac{\text{[3H]NE IR (dpm/min)}}{\text{[3H]NE concentration (dpm/l)}}
\]

where [3H]NE IR is the [3H]NE infusion rate, and

\[
\text{SNESO (nmol/min)} = \frac{\text{[3H]NE IR (dpm/min)}}{\text{NE SA (dpm/nmol)}}
\]

where NE SA is the norepinephrine specific activity. The forearm NE metabolic clearance rate (FNEMCR) and spillover rate (FNESO) (11, 29) were calculated from forearm plasma flow (FPF = forearm blood flow(1 – hematocrit) in ml·min⁻¹·100 ml tissue⁻¹) and forearm fractional extraction of NE (Fex [3H]NE)

\[
F_{\text{ex}} \cdot [3H] \text{NE (unitless)} = \left( \frac{[3H] \text{NE}_A}{[3H] \text{NE}_V} \right) \times \frac{\text{FPF}}{\left( [NE_V - NE_A] + (NE_A \times F_{\text{ex}} \cdot [3H] \text{NE}) \right) \times FPF}
\]

where the subscripts A and V indicate arterial and venous, respectively. Hence

\[\text{FNEMCR (ml·min}^{-1}·100\text{ml tissue}^{-1}) = F_{\text{ex}} \cdot [3H] \text{NE} \times FPF \]

\[\text{FNESO (nmol·min}^{-1}·100\text{ml tissue}^{-1}) = \left( [NE_V - NE_A] + (NE_A \times F_{\text{ex}} \cdot [3H] \text{NE}) \right) \times FPF \]

Forearm blood flow was measured by venous occlusion plethysmography (Parks Medical Electronics, Aloha, OR; see Ref. 21) at the 20-, 25-, and 30-min time points during each [3H]NE infusion along with measurements of NE mass and radioactivity at the same time points. To exclude the hand from the measurement of blood flow, the wrist cuff was inflated to ~230 mmHg for 2 min before recordings and was maintained during the recordings. Each blood flow value was the mean of five consecutive recordings.

In study 1, after instrumentation and a 30-min rest period, [3H]NE was infused for 30 min, there was a 30-min washout period, and [3H]NE was again infused for 30 min. Isotopic steady state is achieved after <20 min (32). On one occasion the subject remained supine throughout; on the other occasion the subject was supine during the first [3H]NE infusion and standing during the second infusion.

In study 2, again after instrumentation and a 30-min rest period, [3H]NE was infused over 30 min three times with 30-min washout periods between infusions, on three occasions in random sequence: one time with saline infusion through all three segments (T1, T2, and T3); one time with saline infusion in T1 and insulin infusion (12.0 pmol·kg⁻¹·min⁻¹) in T2 and T3 with euglycemia (~4.6 mmol/l) maintained by variable 20% glucose infusion through T2 and T3 (43); and one time with saline infusion in T1, insulin infusion in T2 and T3, euglycemia (~4.6 mmol/l) in T2, and hypoglycemia (~2.8 mmol/l) in T3 (43).

Analytical methods. Plasma glucose was measured with a glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Plasma NE and epinephrine concentrations were measured with a single isotope derivative (radioenzymatic) method (44), and those of insulin (28), C-peptide (28), glucagon (15), pancreatic polypeptide (20), cortisol (18), and growth hormone (42) were measured with radioimmunoassays. Serum nonesterified fatty acid levels were measured with an enzymatic colorimetric method.
(26), and blood β-hydroxybutyrate (37), lactate (30), and alanine (7) levels were measured with enzymatic techniques. Neurogenic (autonomic) and neuroglycopenic symptom scores were determined as described previously (43, 52).

Statistical methods. Contrasts over time (T1 vs. T2 in study 1 and T1 vs. T2 in study 2) within each study limb (i.e., supine to supine day and supine to prolonged standing day in study 1 and saline infusion day (control limb), the saline then insulin and glucose infusion day (euglycemic limb), and the saline then insulin then hypoglycemia day (euglycemic to hypoglycemic limb) in study 2) were analyzed by t-test for paired data. Contrasts between limbs were analyzed by general linear models procedure repeated measures analysis of variance (ANOVA) for limb \times time interactions. In this report, P values for time contrasts are shown without further notation, and those for limb \times time interactions are shown with the notation ANOVA. P values <0.05 were considered to indicate statistically significant differences. Data are expressed as means ± SE except where the SD is specified.

RESULTS

Study 1. Mean blood pressures, which did not change over time in the T1 and T2 time segments in the supine to supine limb, decreased from 93 ± 8 mmHg to 69 ± 6 mmHg in T2 (P < 0.02) in the supine to prolonged standing limb (Fig. 1). There was a significant limb \times time interaction (ANOVA, P < 0.02). There were no changes in heart rates (Fig. 1). Plasma pancreatic polypeptide concentrations, which did not change over time in the supine to supine limb, increased from 0.72 ± 0.29 to 1.53 ± 0.26 nmol/l (P < 0.01) in the supine to prolonged standing limb (Fig. 3). There was a significant limb \times time interaction (ANOVA, P < 0.01). Plasma NE concentrations, which did not change in the supine to supine limb, increased from 0.72 ± 0.12 to 1.53 ± 0.26 nmol/l (P < 0.01) in the prolonged standing limb (Fig. 3). There was a significant limb \times time interaction (ANOVA, P < 0.01).

Forearm blood flows, which did not change over time in the supine to supine limb, decreased from 1.79 ± 0.29 to 0.72 ± 0.21 ml·min\(^{-1}\)·100 ml tissue\(^{-1}\) (P < 0.05) in the supine to prolonged standing limb (Fig. 2). There was a significant limb \times time interaction (ANOVA, P < 0.05). Plasma epinephrine concentrations, which did not change over time in the supine to supine limb, decreased from 93 ± 6 mmHg in T1 to 69 ± 6 mmHg in T2 (P < 0.02) in the supine to prolonged standing limb (Fig. 1). There was a significant limb \times time interaction (ANOVA, P < 0.02). There were no changes in heart rates (Fig. 1). Plasma pancreatic polypeptide concentrations, which did not change over time in the supine to supine limb, increased from 0.72 ± 0.29 to 1.53 ± 0.26 nmol/l (P < 0.01) in the supine to prolonged standing limb (Fig. 3). There was a significant limb \times time interaction (ANOVA, P < 0.01). Plasma NE concentrations, which did not change in the supine to supine limb, increased from 0.72 ± 0.12 to 1.53 ± 0.26 nmol/l (P < 0.01) in the prolonged standing limb (Fig. 3). There was a significant limb \times time interaction (ANOVA, P < 0.01).

NE SA were stable at the 20-, 25-, and 30-min sampling times with the subjects in the supine and in the standing positions [data not shown but as also documented previously (30)]. Thus mean data from these three samples were used to calculate NE kinetic values during the final 10 min of each 30-min \(^{3}H\)NE infusion. Systemic NE spillover rates (SNESO), which did not change over time in the supine to supine limb, increased from 2.16 ± 0.39 to 4.62 ± 1.29 nmol/min.
increase (0.58 ± 0.20 to 0.50 ± 0.21 pmol min⁻¹ 100 ml tissue⁻¹) in the prolonged standing limb (Fig. 4). There was a significant limb × time interaction (ANOVA, P < 0.05). However, FNESO, which did not change over time in the supine to supine limb, did not increase (0.58 ± 0.20 to 0.50 ± 0.21 pmol min⁻¹ 100 ml tissue⁻¹) in the prolonged standing limb (Fig. 4). SNEMCR and FNEMCR (data not shown) did not change significantly over time in either limb, and there was no significant limb × time interaction.

Study 2. Plasma glucose concentrations were stable through the T₁, T₂, and T₃ segments in the control (saline infusion) limb, through the T₁ (saline) and the T₂ and T₃ (hyperinsulinemic euglycemia) time segments in the euglycemic limb, and during the T₁ (saline) and T₂ (hyperinsulinemic euglycemia) time segments of the euglycemic to hypoglycemic limb of the study (Fig. 5). Plasma glucose levels were decreased from 4.7 ± 0.1 mmol/l during the T₂ time segment to 2.9 ± 0.1 mmol/l in the T₃ time segment of the latter limb of the study (Fig. 5). Plasma insulin concentrations, which did not change over time in the control limb, were raised comparably during the T₂ and T₃ time segments in both the euglycemic and the euglycemic to hypoglycemic limbs (Table 1). There was not a significant limb × time interaction. Some of the insulin values were below the detection limit of 24 pmol/l and were assigned that value for calculation of the means. Plasma C-peptide concentrations, which did not change over time in the control limb, decreased during the T₂ (P < 0.05) and T₃ (P < 0.001) time segments in the euglycemic limb and the T₂ (P < 0.05) time segment in the euglycemic to hypoglycemic limb (Table 1). C-peptide levels decreased further during hypoglycemia (T₃ time segment in the euglycemic to hypoglycemic limb) compared with during euglycemic hyperinsulinemia (ANOVA, P < 0.01).

Mean blood pressures, which did not change in the control and euglycemic limbs, decreased slightly in the euglycemic to hypoglycemic limb (P < 0.05; Table 2). There was a significant limb × time interaction (ANOVA, P < 0.05). Systolic and diastolic blood pressure patterns were similar (Table 2), again with significant limb × time interactions (both ANOVA, P < 0.05). Heart rates did not change significantly in any of the limbs (Table 2). Rates of forearm blood flow, which did not change during the control and the euglycemic limbs, increased from 1.48 ± 0.31 to 2.52 ± 0.49 ml·min⁻¹·100 ml tissue⁻¹ (P < 0.05) in the T₃ time segment in the euglycemic to hypoglycemic limb (Fig. 6). The increase in forearm blood flow was greater in the euglycemic to hypoglycemic limb than in the euglycemic limb (ANOVA, P < 0.01) despite comparable hyperinsulinemia (Table 1).

Plasma epinephrine concentrations, which did not change in the control and euglycemic limbs, increased from 190 ± 70 to 1,720 ± 320 pmol/l (P < 0.0001) in the T₃ time segment in the euglycemic to hypoglycemic limb (Fig. 7). The increase in plasma epinephrine levels was greater in the euglycemic to hypoglycemic limb than in the euglycemic limb (ANOVA, P < 0.01). Plasma NE concentrations, which were unchanged in the control limb and did not increase significantly in the euglycemic limb, increased from 1.04 ± 0.15 to 1.57 ± 0.18 nmol/l (P < 0.05) in the T₃ time segment in the euglycemic to hypoglycemic limb (Fig. 8). However, the T₁ to T₃ increase in plasma NE levels in the euglycemic to hypoglycemic limb was not significantly greater than the apparent increase in the euglycemic limb (ANOVA, P = 0.11), nor was the T₂ to T₃ increase (ANOVA, P = 0.05).

In addition to decrements in endogenous insulin secretion, hypoglycemia elicited increments in plasma pancreatic polypeptide, glucagon, growth hormone, and cortisol concentrations (Table 1). Increments in each of these were significantly greater in the euglycemic to hypoglycemic limb than in the euglycemic limb (ANOVA, P < 0.001 for pancreatic polypeptide and glucagon and P < 0.05 for growth hormone and cortisol). Serum
nonesterified fatty acid levels were suppressed during hyperinsulinemia in both the euglycemic (P < 0.001) and the euglycemic to hypoglycemic (P < 0.0001) limbs (Table 3). Blood β-hydroxybutyrate patterns were similar (Table 3). Blood lactate concentrations, which did not change in the control limb and appeared to increase in the euglycemic limb, increased during the T2 time segment (P < 0.001) and the T3 time segment (P < 0.02) in the euglycemic to hypoglycemic limb (Table 3). Blood alanine concentrations did not change in any of the limbs (Table 3).

### Table 1. Plasma insulin, C-peptide, pancreatic polypeptide, glucagon, growth hormone, and cortisol concentrations

<table>
<thead>
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<th>Parameter</th>
<th>Time Segment</th>
<th>Study Limb</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Eu</td>
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<tr>
<td>Insulin, pmol/l</td>
<td>T1</td>
<td>24 ± 0</td>
<td>30 ± 6</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>24 ± 0</td>
<td>588 ± 96a</td>
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<td></td>
<td>T3</td>
<td>24 ± 0</td>
<td>602 ± 86a</td>
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<td>C-peptide, nmol/l</td>
<td>T1</td>
<td>0.43 ± 0.03</td>
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<td></td>
<td>T2</td>
<td>0.40 ± 0.07</td>
<td>0.33 ± 0.10b</td>
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<td>T3</td>
<td>0.33 ± 0.03</td>
<td>0.23 ± 0.07a</td>
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<td>Pancreatic polypeptide, pmol/l</td>
<td>T1</td>
<td>24 ± 8</td>
<td>31 ± 12</td>
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<td></td>
<td>T2</td>
<td>22 ± 6</td>
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<td></td>
<td>T3</td>
<td>37 ± 20</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Glucagon, pmol/l</td>
<td>T1</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>17 ± 1</td>
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<td></td>
<td>T3</td>
<td>17 ± 1</td>
<td>14 ± 1c</td>
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<td>Growth hormone, pmol/l</td>
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<td>93 ± 31</td>
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<td>T2</td>
<td>181 ± 49</td>
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<td></td>
<td>T3</td>
<td>115 ± 26</td>
<td>490 ± 203</td>
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<td>Cortisol, nmol/l</td>
<td>T1</td>
<td>280 ± 70</td>
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<td>320 ± 50</td>
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<tr>
<td></td>
<td>T3</td>
<td>300 ± 20</td>
<td>470 ± 180</td>
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Mean ± SE plasma insulin, C-peptide, pancreatic polypeptide, glucagon, growth hormone, and cortisol concentrations in time segments T1, T2, and T3 during the control (saline throughout) limb, the euglycemic (Eu) limb (saline in T1; insulin in T2 and T3), and the euglycemic to hypoglycemic (Eu→Hypo) limb (saline in T1; insulin in T2 and T3; hypoglycemia in T3) in healthy humans in study 2. To convert insulin to µU/ml divide by 6.0, C-peptide to ng/ml divide by 0.331, pancreatic polypeptide to pg/ml divide by 0.239, glucagon to pg/ml divide by 0.2871, growth hormone to ng/ml divide by 44.15, and cortisol to µg/dl divide by 27.59. *P < 0.0001 vs. T1; bP < 0.05 vs. T1; cP < 0.01 vs. T1; dP < 0.001 vs. T1; eP < 0.002 vs. T1.

### Table 2. Mean, systolic, and diastolic blood pressure and heart rate

<table>
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<th>Parameter</th>
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<th>Study Limb</th>
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<tr>
<td>Mean blood pressure, mmHg</td>
<td>T1</td>
<td>91 ± 6</td>
<td>90 ± 5</td>
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<td>90 ± 3</td>
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<td></td>
<td>T3</td>
<td>98 ± 4</td>
<td>100 ± 9</td>
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<td>Systolic blood pressure, mmHg</td>
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<td>146 ± 8</td>
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<td>T3</td>
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<td>Diastolic blood pressure, mmHg</td>
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<td>T2</td>
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<td></td>
<td>T3</td>
<td>68 ± 4</td>
<td>74 ± 10</td>
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<td>Heart rate, beats/min</td>
<td>T1</td>
<td>62 ± 8</td>
<td>57 ± 6</td>
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<td></td>
<td>T2</td>
<td>61 ± 8</td>
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<td>T3</td>
<td>62 ± 8</td>
<td>66 ± 8</td>
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Data are means ± SE for mean, systolic, and diastolic blood pressures and heart rates in segments T1, T2, and T3 during the control (saline throughout) limb, the euglycemic limb (saline in T1; insulin in T2 and T3), and the euglycemic to hypoglycemic limb (saline in T1; insulin in T2 and T3; hypoglycemia in T3) in healthy humans in study 2. *P < 0.05 vs. T1.

**Fig. 6.** Mean ± SE forearm blood flow in the final 10 min of the T1, T2, and T3 segments in the control limb, the euglycemic limb, and the euglycemic to hypoglycemic limb in healthy subjects during saline infusions (open bars) and insulin infusions with euglycemia (cross-hatched bars) or hypoglycemia (filled bar) in study 2. *Significant difference from the baseline value on the same occasion.
conditions in greater than those in SNESO under the various study
the three limbs (data not shown). Neuroglycopenic symptom
scores did not change significantly over time in any of
(other data not shown). Neuroglycopenic symptom

significantly greater than that in the euglycemic limb (ANOVA,
P = 0.14), the T2 to T3 increase was greater in the
euglycemic to hypoglycemic limb (ANOVA, P < 0.05).
FNESO, which did not change in the control limb,
increased from 0.57 ± 0.11 to 1.25 ± 0.25 pmol·min⁻¹·100 ml tissue⁻¹ (P < 0.05) in the euglycemic limb and
from 0.36 ± 0.08 to 1.03 ± 0.37 pmol·min⁻¹·100 ml
tissue⁻¹ (P < 0.05) in the euglycemic to hypoglycemic
limb (Fig. 10). The increase in forearm NE spillover
was not significantly greater in the euglycemic to
hypoglycemic limb than in the euglycemic limb.
SNEMCR and FNEMCR did not change over time in
any of the three limbs (data not shown). Neurogenic
symptom scores did not change in the control and
euglycemic limbs, increased from 1.2 ± 0.4 to 4.0 ±
0.9 (P < 0.02) in the euglycemic to hypoglycemic limb
(other data not shown). Neuroglycopenic symptom
scores did not change significantly over time in any of
the three limbs (data not shown).

Relative increments in FNESO were consistently
greater than those in SNESO under the various study
conditions in study 2. During hyperinsulinemic euglyce-

Fig. 7. Mean ± SE plasma epinephrine concentrations at the end of
the T1, T2, and T3 segments in the control limb, the euglycemic limb,
and the euglycemic to hypoglycemic limb in healthy subjects during
saline infusions (open bars) and insulin infusions with euglycemia
(crosshatched bars) or hypoglycemia (filled bar) in study 2. *Signifi-
cant difference from the baseline value on the same occasion.

FNESO in the euglycemic limb of the study the increases
were 1.9- vs. 1.4-fold, respectively, from the T1 to the T2
segment and 2.2- and 1.4-fold, respectively, from the T1
to the T3 segment; in the euglycemic to hypoglycemic
limb they were 2.0- vs. 1.3-fold, respectively, from the
T1 to the T2 segment. During hyperinsulinemic hypogly-
cemia in the euglycemic to hypoglycemic limb the
increases were 2.8- vs. 1.6-fold, respectively, from the
T1 to the T2 segment and 1.4- vs. 1.2-fold in the T2 to the
T3 Segment.

DISCUSSION

We used measurements of FNESO, coupled with
those of SNESO and plasma NE and epinephrine
concentrations, to distinguish sympathetic neural from
adrenomedullary activation during prolonged stand-

Fig. 8. Mean ± SE plasma NE concentrations at the end of the T1, T2,
and T3 segments in the control limb, the euglycemic limb, and the
euglycemic to hypoglycemic limb in healthy subjects during saline
infusions (open bars) and insulin infusions with euglycemia (cross-
hatched bars) or hypoglycemia (filled bar) in study 2. *Signifi-
cant difference from the baseline value on the same occasion.

Fig. 9. Mean ± SE systemic NE spillover rates during the last 10 min
of the T1, T2, and T3 segments in the control limb, the euglycemic
limb, and the euglycemic to hypoglycemic limb in healthy subjects
during saline infusions (open bars) and insulin infusions with
euglycemia (crosshatched bars) or hypoglycemia (filled bar) in study
2. *Significant difference from the baseline value on the same
occasion.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Segment</th>
<th>Control</th>
<th>Eu</th>
<th>Eu—Hypo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonesterified fatty acids, μmol/l</td>
<td>T1</td>
<td>789 ± 20</td>
<td>554 ± 88</td>
<td>689 ± 72</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>814 ± 92</td>
<td>147 ± 36</td>
<td>134 ± 60</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>649 ± 74</td>
<td>138 ± 32a</td>
<td>78 ± 36a</td>
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<tr>
<td>β-Hydroxybutyrate, μmol/l</td>
<td>T1</td>
<td>194 ± 61</td>
<td>340 ± 169</td>
<td>275 ± 95</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>214 ± 60</td>
<td>109 ± 13</td>
<td>117 ± 21</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>246 ± 45</td>
<td>123 ± 16</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>Lactate, μmol/l</td>
<td>T1</td>
<td>637 ± 198</td>
<td>820 ± 222</td>
<td>658 ± 142</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>574 ± 112</td>
<td>1,266 ± 175</td>
<td>1,291 ± 101a</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>576 ± 133</td>
<td>1,183 ± 102</td>
<td>1,245 ± 190c</td>
</tr>
<tr>
<td>Alanine, μmol/l</td>
<td>T1</td>
<td>317 ± 17</td>
<td>332 ± 73</td>
<td>308 ± 45</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>291 ± 26</td>
<td>306 ± 54</td>
<td>329 ± 26</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>271 ± 23</td>
<td>304 ± 27</td>
<td>324 ± 40</td>
</tr>
</tbody>
</table>

Mean ± SE serum nonesterified fatty acid and blood β-hydroxybuty-
rate, lactate, and alanine concentrations in segments T1, T2, and T3
during the control (saline throughout) limb, the euglycemic limb
(saline in T3, insulin in T2 and T3), and the euglycemic to hypoglyce-
mic limb (saline in T1, insulin in T2 and T3, hypoglycemia in T3) in
healthy humans in study 2. *P < 0.001 vs. T1; bP < 0.0001 vs. T3; cP <
0.02 vs. T1.
The data suggest that the raised plasma NE concentrations and SNESO, but not FNESO, are consistent with that construct. Taken at face value the data support the idea that increased NE release from sympathetic nerves other than those in the forearm (16) (and to some extent from the adrenal medullas given the observed 5-fold increase in the plasma epinephrine concentration). Thus hyperinsulinemia per se stimulates the sympathetic neural but not the adrenomedullary component of the sympathochromaffin system, and that while hypoglycemia per se stimulates the adrenomedullary component it may not stimulate the sympathetic neural component.

Prolonged (30–60 min) standing was associated with decrements in systolic and diastolic (and therefore mean) blood pressures and forearm blood flow without an increase in heart rate, a pattern suggesting that sympathetic neural activity was no longer increased substantially, and parasympathetic neural activity was increased, in healthy young adults. This hemodynamic pattern is that of the vasodepressor (vasovagal, neurocardiogenic) response that can lead to syncope (53). There is considerable evidence, based on measurements of plasma NE concentrations (21, 41, 48, 58), cardiac and renal NE spillover rates (16), and microneurography (25, 35, 36, 57), that a decrease in sympathetic neural activity is a key component of the vasodepressor response. The present findings of increments in plasma NE concentrations and SNESO, but not FNESO, are consistent with that construct. Taken at face value the data suggest that the raised plasma NE concentrations and SNESO were the result of increased NE release from sympathetic nerves other than those in the forearm (16) (and to some extent from the adrenal medullas given the observed 5-fold increase in the plasma epinephrine concentration). In addition, the finding of increased plasma pancreatic polypeptide levels, a putative marker of pancreatic parasympathetic outflow (41), could explain the absence of an increase in heart rate if parasympathetic outflow to the heart parallels that to the pancreas. However, at the time of incipient vasodepressor syncope, when the subjects were studied, they still had measurable, albeit reduced, blood pressures and had not lost consciousness. Therefore, although likely less than shortly after standing, (6, 45, 51), net sympathetic neural activity must have been increased to some extent.

The assumption that infused labeled NE mixes with a constant fraction of NE released at sympathetic nerve terminals, which is implicit in the application of the clearance concept to the quantification of NE kinetics, has been challenged by Christensen and Knudsen (10). If this condition is not met when the capillary surface exchange area is increased (14) or decreased, the plasma NE spillover rate would be underestimated or overestimated, respectively. This might explain a reported discrepancy between muscle sympathetic nerve activity measured with microneurography and plasma NE concentrations (both increased) and plasma NE spillover (unchanged) during lower body negative pressure, a condition in which the capillary surface exchange area would be expected to be decreased (10). The latter would also be expected during prolonged standing in the present study; our methods did not include a measure of capillary surface exchange area (14). Thus the FNESO could have been underestimated. Finally, with respect to such technical issues, the calculated forearm NE plasma appearance rate, which includes an extraction term, has been reported to be influenced to a lesser degree by local changes in NE clearance and blood flow (7) than the spillover rate (8). However, the pattern of findings reported here was the same when expressed as the appearance rate, albeit with larger variances, as it was when expressed as the spillover rate in both study 1 and study 2.

Compared with the plasma NE concentration and the SNESO, the FNESO was more variable when measured three times with the subjects in the supine position, perhaps because of variation in the measurement of forearm blood flow.

To assess the extent to which increments in plasma NE concentrations during hyperinsulinemia and hypoglycemia are the result of sympathetic neural activation, adrenomedullary activation, or both, subjects were studied (in the supine position) during saline infusion, hyperinsulinemic euglycemia, and hyperinsulinemic hypoglycemia. Euglycemic hyperinsulinemia caused increments in FNESO and apparent increments in SNESO and plasma NE levels, with no change in plasma epinephrine concentrations. Thus hyperinsulinemia per se stimulates the sympathetic neural, but not the adrenomedullary, component of the sympathochromaffin system. This pattern, a significant increment in FNESO without significant increments in plasma NE or SNESO, suggests that the forearm NE spillover measurement, like microneurography (22), is a more sensitive measure of sympathetic neural activation than the plasma NE concentration (or the SNESO) at least under this condition.

Our finding of unaltered plasma epinephrine concentrations during hyperinsulinemic euglycemia is consistent with most, but not all, previous studies. For example, Tack et al. (49) found 90 min of hyperinsulinemic euglycemia to be associated with statistically significant increments in arterial plasma epinephrine concentrations. However, the increments were small.

Fig. 10. Mean ± SE forearm NE spillover rates during the last 10 min of the T1, T2, and T3 segments in the control limb, the euglycemic limb, and the hypoglycemic limb in healthy subjects during saline infusions (open bars) and insulin infusions with euglycemia (crosshatched bars) or hypoglycemia (filled bar) in study 2. *Significant difference from the baseline value on the same occasion.
Plasma epinephrine concentrations (and forearm blood flows) did not change during hyperinsulinemic euglycemia but increased substantially during hyperinsulinemic hypoglycemia. Clearly, hypoglycemia per se stimulates adrenomedullary activity. Despite an apparent stepwise increase from the T1 (saline) to the T2 (hyperinsulinemia euglycemia) to the T3 (hyperinsulinemic hypoglycemia), increments in plasma NE concentrations from T1 to T3 (ANOVA, \( P = 0.11 \)) and from T1 to T3 (ANOVA, \( P = 0.05 \)) were not significantly greater than those in the euglycemic limb. Similarly, despite an apparent stepwise increase from the T1 to the T2 to the T3 time segments in the euglycemic to hypoglycemic limb, increments in SNESO from T1 to T3 were not significantly greater than those in the euglycemic limb (ANOVA, \( P = 0.14 \)), although the increments from T2 to T3 were significantly greater (ANOVA, \( P < 0.05 \)). Although the latter finding suggests greater NE release during hyperinsulinemic hypoglycemia than during comparably hyperinsulinemic euglycemia, it does not identify the source. Given substantial adrenomedullary stimulation, evidenced by a ninefold increase in plasma epinephrine concentrations, it is conceivable that the additional NE was derived from the adrenal medullas. The forearm NE spillover data are consistent with that interpretation. Neither the absolute values nor the increments in FNESO were greater in the euglycemic to hypoglycemic limb than in the euglycemic limb. Thus the present data do not provide direct support for the concept that hypoglycemia per se also stimulates the sympathetic neural component of the sympathochromaffin system. As in study 1, there was considerable scatter in the forearm NE spillover data in study 2. That, and our small sample sizes, might have obscured differences between the magnitude of the responses to hyperinsulinemic hypoglycemia and euglycemia. Nonetheless, although the present data do not rule out the possibility that hypoglycemia per se stimulates sympathetic neural activity in sites other than the forearm, they are consistent with the possibility that hypoglycemia per se stimulates the adrenal medullas and not the sympathetic nervous system.

To our knowledge, all studies of sympathetic neural responses to hypoglycemia have employed insulin-induced hypoglycemia and are, therefore, potentially confounded by insulin-stimulated sympathetic neural activity. We are not aware of published studies, using microelectrode, regional NE spillover, or microdialysis to measure sympathetic neural activity separately from adrenomedullary activity, that have contrasted sympathetic neural activity during hyperinsulinemic euglycemia and hyperinsulinemic hypoglycemia over the same time frame as was done in the present study. However, using microneurography, Frandsen et al. (19) found increments in muscle sympathetic nerve activity during hyperinsulinemic euglycemic clamps and further increments during subsequent hypoglycemic clamps at the same insulin infusion rate in healthy human subjects.

Hyperinsulinemia per se lowered plasma C-peptide and glucagon levels, raised blood lactate levels, and suppressed nonesterified fatty acid levels. Hyperinsulinemic hypoglycemia lowered plasma C-peptide levels further and raised plasma glucagon, epinephrine, NE, pancreatic polypeptide, growth hormone and cortisol levels, and SNESO and FNESO. Despite adrenomedullary activation and increased growth hormone and cortisol secretion, serum nonesterified fatty acid levels remained suppressed, an effect attributable to the potent antilipolytic action of ongoing hyperinsulinemia and the relatively short duration of activation of these lipolytic factors.

Although it is a simple and often useful index, the plasma NE concentration is a fallible index of sympathetic neural activity because circulating NE can be derived from sympathetic nerve terminals, the adrenal medullas, or both and because of regional differences in sympathetic neural activity under various conditions. Albeit in the uncommon circumstance of prolonged standing, the present data indicate that increments in plasma NE concentrations (and SNESO) can be dissociated from sympathetic neural activity in one region, the forearm, as assessed by the FNESO. Furthermore, the present data confirm that hyperinsulinemia per se stimulates sympathetic neural activity (1, 2, 4, 5, 9, 29, 39, 47, 50, 55), here assessed with the FNESO, without stimulating adrenomedullary activity, an issue that is still debated (33). Finally, although they confirm that hypoglycemia per se activates adrenomedullary activity (45), the present data do not provide direct support for the concept that hypoglycemia per se stimulates sympathetic neural activity (3, 4, 17, 31). Increments in forearm NE spillover during hyperinsulinemic hypoglycemia were not significantly greater than those during hyperinsulinemic euglycemia.

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REFERENCES


