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. Furthermore, when these data do not provide direct support for the concept that hypoglycemia per se also stimulates sympathetic neural activity.

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 fractional rate (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 fractional rate 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

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

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

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

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)

\[
F_{\text{ex}} [3H] \text{NE} \text{ (unitless)} = \frac{[3H] \text{NE}_{A} - [3H] \text{NE}_{V}}{[3H] \text{NE}_{A}}
\]

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

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

\[
\text{FNESO (nmol·min⁻¹·100 ml tissue⁻¹)} = (NE_{V} - NE_{A}) + (NE_{A} \times F_{\text{ex}} [3H] \text{NE}) \times FPF
\]

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., the supine to supine limb and the supine to prolonged standing limb in study 1 and the 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 × time interactions. In this report, P values for time contrasts are shown without further notation, and those for limb × 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 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 × 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 11 ± 2 to 42 ± 9 pmol/l (P < 0.01) in the supine to prolonged standing limb (Fig. 2). There was a significant limb × time interaction (ANOVA, P < 0.01). Forearm blood flows, which did not change over time in the supine to supine limb, fell 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 × 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 to 62 ± 13 pmol/l (P < 0.01) in the supine to prolonged standing limb (Fig. 3). There was a significant limb × 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 × 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 [3H]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

Fig. 1. Mean ± SE blood pressures (A) and heart rates (B) in healthy subjects with the subjects supine (Su, open bars) during two consecutive periods separated by 30 min on one occasion and supine and then standing (St, filled bars) for 30–60 min on another occasion in study 1. *Significant difference from the baseline value on the same occasion.

Fig. 2. Mean ± SE plasma pancreatic polypeptide concentrations (A) and forearm blood flow rates (B) in healthy subjects with the subjects supine (open bars) during two consecutive periods separated by 30 min on one occasion and supine and then standing (filled bars) for 30–60 min on another occasion in study 1. *Significant differences from the baseline values on the same occasion.

Fig. 3. Mean ± SE plasma epinephrine concentrations (A) and plasma norepinephrine (NE) concentrations (B) in healthy subjects with the subjects supine (open bars) during two consecutive periods separated by 30 min on one occasion and supine and then standing (filled bars) for 30–60 min on another occasion in study 1. *Significant differences from the baseline values on the same occasion.
(P < 0.05) in the supine to 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 < 0.01 for pancreatic polypeptide and glucagon and P < 0.05 for growth hormone and cortisol). Serum

![Fig. 4](http://ajpendo.physiology.org/)
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 rate 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>
<tr>
<th>Parameter</th>
<th>Time Segment</th>
<th>Control</th>
<th>Eu</th>
<th>Eu→Hypo</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/l</td>
<td>T1</td>
<td>24 ± 0</td>
<td>30 ± 6</td>
<td>24 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>24 ± 0</td>
<td>588 ± 96a</td>
<td>552 ± 42a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>24 ± 0</td>
<td>602 ± 86a</td>
<td>615 ± 48a</td>
<td></td>
</tr>
<tr>
<td>C-peptide, nmol/l</td>
<td>T1</td>
<td>0.43 ± 0.03</td>
<td>0.53 ± 0.07</td>
<td>0.53 ± 0.07</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>T2</td>
<td>0.40 ± 0.07</td>
<td>0.33 ± 0.010</td>
<td>0.33 ± 0.070</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.33 ± 0.03</td>
<td>0.23 ± 0.07</td>
<td>0.07 ± 0.009</td>
<td>&lt;0.01</td>
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<tr>
<td>Pancreatic polypeptide, pmol/l</td>
<td>T1</td>
<td>24 ± 8</td>
<td>31 ± 12</td>
<td>22 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>22 ± 6</td>
<td>16 ± 5</td>
<td>16 ± 2</td>
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<tr>
<td></td>
<td>T3</td>
<td>37 ± 20</td>
<td>21 ± 3</td>
<td>79 ± 17a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucagon, pmol/l</td>
<td>T1</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
<td>18 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>17 ± 1</td>
<td>14 ± 1e</td>
<td>13 ± 1e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>17 ± 1</td>
<td>14 ± 1e</td>
<td>74 ± 2e</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Growth hormone, pmol/l</td>
<td>T1</td>
<td>93 ± 31</td>
<td>371 ± 260</td>
<td>62 ± 9</td>
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<tr>
<td></td>
<td>T2</td>
<td>161 ± 49</td>
<td>446 ± 349</td>
<td>57 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>115 ± 26</td>
<td>490 ± 203</td>
<td>1,060 ± 30a</td>
<td>&lt;0.05</td>
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<tr>
<td>Cortisol, nmol/l</td>
<td>T1</td>
<td>280 ± 70</td>
<td>360 ± 90</td>
<td>400 ± 90</td>
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<tr>
<td></td>
<td>T2</td>
<td>260 ± 50</td>
<td>320 ± 50</td>
<td>370 ± 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>300 ± 20</td>
<td>470 ± 180</td>
<td>720 ± 100a</td>
<td>&lt;0.05</td>
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</table>

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 divided 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; **P < 0.05 vs. T1; ***P < 0.001 vs. T1; ****P < 0.01 vs. T1; and ***P < 0.02 vs. T1.

NE SA were stable at the 20-, 25-, and 30-min sampling times in all three limbs (data not shown). Thus mean data from these three samples were used to calculate NE kinetic values during the final 10 min of each 30-min [14C]NE infusion in each time segment of each limb. SNESO, which did not change in the control limb and did not increase significantly in the euglycemic limb, increased from 3.45 ± 0.69 to 5.59 ± 0.88 nmol/min (P < 0.05) in the euglycemic to hypoglycemic limb (Fig. 9). Although the T1 to T3 increase in SNESO in the euglycemic to hypoglycemic limb was not signifi-
from 0.36  

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). Neurogenic  
tissue  

5  
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, which did not change in the control  
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.

Relative increments in FNESO were consistently  
greater than those in SNESO under the various study  
conditions in study 2. During hyperinsulinemic euglycemic  
limb 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 T3 segment and 2.8- vs. 1.6-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-  
ing, hyperinsulinemic euglycemia, and hyperinsulin-

**Table 3. Nonesterified fatty acid, β-hydroxybutyrate, lactate, and alanine concentrations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Segment</th>
<th>Study Limb</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>
<td></td>
</tr>
<tr>
<td>β-Hydroxybutyrate, µmol/l</td>
<td>T1</td>
<td>649 ± 74</td>
<td>138 ± 32*</td>
<td>78 ± 36*</td>
<td></td>
</tr>
<tr>
<td>Lactate, µmol/l</td>
<td>T1</td>
<td>214 ± 60</td>
<td>109 ± 13</td>
<td>117 ± 21</td>
<td></td>
</tr>
<tr>
<td>Alanine, µmol/l</td>
<td>T1</td>
<td>291 ± 26</td>
<td>306 ± 54</td>
<td>329 ± 26</td>
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</tbody>
</table>

Mean ± SE serum nonesterified fatty acid and blood β-hydroxybu- 
yturate, lactate, and alanine concentrations 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 hypoglyce- 
mic limb (saline in T1, insulin in T2, and hypoglycemia in T3) in  
healthy humans in study 2. *P < 0.001 vs. T1; bP < 0.0001 vs. T1; *P < 0.02 vs. T1.

**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. *Significant difference from the baseline value on the same occasion.**

**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 (crosshatched bars) or hypoglycemia (filled bar) in study 2. *Significant 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.**
emergence. Therefore, although likely less than shortly after
reduced, blood pressures and had not lost conscious-
subjects were studied, they still had measurable, albeit

time of incipient vasodepressor syncope, when the
heart parallels that to the pancreas. However, at the
increase in heart rate if parasympathetic outflow to the
thetic outflow (41), could explain the absence of an
levels, a putative marker of pancreatic parasympa-
finding of increased plasma pancreatic polypeptide
plasma epinephrine concentration). In addition, the
medullas given the observed 5-fold increase in the
release from sympathetic nerves other than those in

Prolonged (30–60 min) standing was associated with
decrements in systolic and diastolic (and therefore
mean) blood pressures and forearm blood flow without
an increment 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, neuro-
cardiogenic) response that can lead to syncope (53). There is considerable evidence, based on measure-
ments of plasma NE concentrations (21, 41, 48, 58),
cardiac and renal NE spillover rates (16), and microneu-
rography (25, 35, 36, 57), that a decrease in sympa-
thetic neural activity is a key component of the vasode-
pressor response. The present findings of increments in
plasma NE concentrations and SNESO, but not FNESE,
are consistent with that construct. Taken at face value
the data suggest that the raised plasma NE concen-
trations 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 parasympa-
thetic 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 conscious-
ness. 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 overestimated or
underestimated, 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 pres-
sure, a condition in which the capillary surface ex-
change 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 underesti-
mated. 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 mea-
sured three times with the subjects in the supine
position, perhaps because of variation in the measure-
ment of forearm blood flow.

To assess the extent to which increments in plasma
NE concentrations during hyperinsulinemia and hypo-
glycemia are the result of sympathetic neural activa-
tion, adrenomedullary activation, or both, subjects
were studied (in the supine position) during saline
infusion, hyperinsulinemic euglycemia, and hyperinsu-
linemic hypoglycemia. Euglycemic hyperinsulinemia
caused increments in FNESO and apparent incremen-
ts in SNESO and plasma NE levels, with no change in
plasma epinephrine concentrations. Thus hyperinsulin-
emia per se stimulates the sympathetic neural, but not
the adrenomedullary, component of the sympathochro-
maffin 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 concen-
trations during hyperinsulinemic euglycemia is consis-
tent with most, but not all, previous studies. For
example, Tack et al. (49) found 90 min of hyperinsul-
linemic euglycemia to be associated with statistically
significant increments in arterial plasma epinephrine
concentrations. However, the increments were small
(from 240 ± 40 to 340 ± 50 pmol/l); the stimulated levels were probably not high enough to exert biological effects (12, 13).

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) time segments in the euglycemic to hypoglycemic clamp, 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 samples sizes, might have obscured differences between the magnitude of the responses to hyperinsulinemic hypoglycemia and euglycemia. Nonetheless, although the present data do not exclude 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 microelectrodes, 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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