Phenotypical evidence for a gender difference in cardiac norepinephrine transporter function

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Schroeder, Christoph, Frauke Adams, Michael Boschmann, Jens Tank, Sebastian Haertert, Andre Diedrich, Italo Biaggioni, Friedrich C. Luft, and Jens Jordan. Phenotypical evidence for a gender difference in cardiac norepinephrine transporter function. Am J Physiol Regul Integr Comp Physiol 286: R851–R856, 2004. First published January 15, 2004; 10.1152/ajpregu.00689.2003.—Norepinephrine transporter (NET) function has a central role in the regulation of synaptic norepinephrine concentrations. Clinical observations in orthostatic intolerance patients suggest a gender difference in NET function. We compared the cardiovascular response to selective NET inhibition with reboxetine between 12 healthy men and 12 age-matched women. Finger blood pressure, brachial blood pressure, and heart rate were measured. The subjects underwent cardiovascular autonomic reflex testing and a graded head-up tilt test. In a separate study, we applied incremental concentrations of tyramine and isoproterenol through subcutaneous microdialysis catheters in eight men and in eight women. NET inhibition elicited a threefold greater increase in supine blood pressure in men than women (P < 0.05). The pressor response was driven by an increased cardiac output. The orthostatic heart rate increase during NET inhibition was greater in men than in women (56 ± 5 beats/min in men, 42 ± 4 beats/min in women, P < 0.001). In contrast, NET inhibition resulted in a similar suppression in the cold pressor and handgrip response, low-frequency blood pressure oscillations, and venous norepinephrine in the supine position. Men and women were similarly sensitive to the lipolytic effect of isoproterenol and tyramine. We conclude that NET inhibition results in more pronounced changes in cardiac regulation in men than women. Our observations suggest that the NET contribution to cardiac norepinephrine turnover may be decreased in women. The gender difference in NET function may not be expressed in tissues that are less NET dependent than the heart.

autonomic nervous system

THE CARDIOVASCULAR AND METABOLIC effects of norepinephrine are determined by the amount of norepinephrine acting upon adrenoreceptors. The amount of synaptic norepinephrine is influenced by two factors, namely norepinephrine release from adrenergic neurons and norepinephrine removal. The latter is mainly achieved through neuronal reuptake via the norepinephrine transporter (NET). Thus NET has a central role in the regulation of norepinephrine turnover, both in the central nervous system and in peripheral tissues (9). The mechanisms that regulate NET function in humans are poorly understood. Recent studies in orthostatic intolerance patients suggest a gender difference in NET function. Orthostatic intolerance is associated with hyperadrenergic symptoms together with orthostatic tachycardia in the absence of orthostatic hypotension (18). Familial orthostatic intolerance can be caused by a rare functional mutation in the NET gene (28). Decreased norepinephrine clearance (20) and a reduction in the venous dihydroxyphenylglycol (DHPG)-to-norepinephrine ratio (21) are consistent with impaired neuronal norepinephrine uptake in sporadic orthostatic intolerance. Orthostatic intolerance affects predominantly women in their reproductive years (18). The hypothesis that gender, perhaps through sex hormones, may influence NET function is supported by animal studies. In these studies, NET expression and function in different areas of the central nervous system were modulated by testosterone (27), estrogen, and progesterone (34). We tested the hypothesis that NET function is decreased in women. To address this issue, we compared the effect of selective NET inhibition in men and in women. Furthermore, we used the microdialysis technique to assess NET function at the adipose tissue level.

METHODS

Subjects

We tested the effect of systemic NET inhibition in 24 young healthy volunteers (12 women, aged 29 ± 2 yr, body mass index 21 ± 1 kg/m²; 12 men, aged 29 ± 2 yr, 24 ± 1 kg/m²). Women were studied with placebo on day 12.4 ± 2.0 (range 2–22) and with reboxetine on day 11.9 ± 2.4 (2–21) after the last menstrual cycle. The subjects received no medication except for oral contraceptives (11 of 12 women). Another group of normal-weight, healthy young subjects underwent a microdialysis study to characterize adrenergic regulation at the adipose tissue level (8 women, aged 27 ± 0.6 yr, body mass index 21 ± 0.4 kg/m²; 8 men, aged 26 ± 1 yr, body mass index 22 ± 0.8 kg/m²). Women in the microdialysis study were studied between day 7 and day 14 of the menstrual cycle. None ingested oral contraceptives. Written informed consent was obtained before inclusion. All procedures were approved by the institutional review board of the Charité, Berlin, Germany.

Systemic NET Inhibition

Volunteers abstinained from substances that interfere with endogenous catecholamines for at least 48 h before testing. In a double-blind, randomized fashion, test subjects ingested 8 mg of the selective norepinephrine transporter inhibitor reboxetine (Edronax, Pharmacia Upjohn) or placebo 12 h and 1 h before testing. An antecubital intravenous line was placed for blood sampling. A unipolar lead electrocardiogram was continuously recorded. Brachial blood pres-
sure was measured every 3 min with an automated oscillometric device (Dinamap, Critikon) on the right arm. Beat-by-beat finger blood pressure (Finapres, Ohmeda) and thoracic impedance were monitored continuously. The placement of the impedance electrodes was carefully documented to ensure similar conditions on both study days. For isometric handgrip testing, participants squeezed a rubber ball at 30% of maximal voluntary contraction over 3 min. The cold pressor test was performed by ice water immersion of the right hand for 1 min. After 15 min of supine rest a graded head-up tilt test was performed. The tilt angle was increased by 15° every 3 min, until 75° were reached. Blood samples for the determination of plasma catecholamine and reboxetine concentrations (15) were obtained in the supine position. Another blood sample for plasma catecholamines was obtained at the end of head-up tilt testing. Plasma catecholamines were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection as described previously (13).

Signals of finger blood pressure and thoracic impedance were analog to digital converted at 500 Hz using the Windaq pro+ software (Datq Instruments). R-R intervals (time between subsequent R waves in the electrocardiogram), blood pressure, and respiration were defined offline using a program written by Andre Diedrich (Vanderbilt University, Nashville, TN) based on PV-wave software (Visual Numerics). Cardiac stroke volume was calculated according to Sramek’s formula (14, 29). Cardiac output was calculated as stroke volume × heart rate. Systemic vascular resistance was calculated as mean arterial pressure divided by cardiac output. We report relative changes in stroke volume, cardiac output, and systemic vascular resistance.

We calculated the power spectra of systolic blood pressure (SBP) and R-R interval time series using fast fourier transformation (segment length 256 s, resampling with 4 Hz, resolution 0.004 Hz) and the cross spectra between R-R intervals and SBP in the low-frequency (LF) band (7, 31).

### Microdialysis

Microdialysis is a useful method to study the effect of locally applied substances in the absence of systemic effects. Two microdialysis catheters were inserted into subcutaneous adipose tissue at the level of the umbilicus after surface anesthesia (4, 22). We perfused the probes at a flow rate of 2 µl/min with Ringer solution (Serumwerke Bernburg, Bernburg, Germany) supplemented with 50 mM ethanol (for blood flow monitoring). CMA/60 microdialysis catheters and CMA/102 microdialysis pumps (both from CMA Microdialysis AB, Solna, Sweden) were used. After a baseline period of 60 min, one microdialysis catheter was perfused with incremental concentrations of isoproterenol (0.01, 0.1, and 1 µM) (Abbot, Oktigies, France) and the other with incremental tyramine concentrations (0.35 and 3.5 mM) (Clinalfa, Laefulingen, Switzerland) (5, 22). Ethanol concentration was determined in the perfusate (inflow) and dialysate (outflow) using a standard enzymatic assay (2). Dialysate glycerol concentrations were determined as a measure of lipolysis using the CMA/600 analyzer (CMA Microdialysis).

### RESULTS

#### Systemic NET Inhibition

**Reboxetine plasma concentrations.** Venous reboxetine plasma concentrations immediately before testing were 255 ± 28 ng/ml in women and 230 ± 30 ng/ml in men (NS).

**Supine hemodynamics.** With placebo, men had a lower resting heart rate than women (Table 1, Fig. 1). NET inhibition increased supine heart rate similarly in men and women (6 ± 3 beats/min in women and 6 ± 2 beats/min in men). With NET inhibition, blood pressure increased 14 ± 3/5 ± 2 mmHg in men and 5 ± 3/2 ± 1 mmHg in women (P < 0.05 for SBP, Fig. 2). In women, supine cardiac output did not change with reboxetine (+5 ± 8% on reboxetine day compared with placebo). Women had a lower supine heart rate, systolic blood pressure, and diastolic blood pressure than men. Reboxetine increased heart rate and atrial fibrillation in both genders. With reboxetine, heart rate was strongly correlated with atrial fibrillation (r = 0.83, P < 0.001).

#### Upright hemodynamics

In the upright position, men had a higher heart rate, systolic blood pressure, diastolic blood pressure, and systemic vascular resistance than women. Reboxetine did not affect heart rate or blood pressure. With reboxetine, systemic vascular resistance decreased by 15 mmHg in women (5%); however, this decrease was not statistically significant.

### Statistics

All data are expressed as means ± SE. Intraand individual and interindividual differences were compared by paired and unpaired t-tests, respectively. Nonparametric data were analyzed by Wilcoxon matched-pairs test or Mann-Whitney test. ANOVA testing for repeated measures was used for multiple comparisons. Relationships between parameters were assessed by linear regression analysis. A value for P < 0.05 was considered significant.

### Table 1. Supine and upright hemodynamics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Reboxetine</th>
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<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>71 ± 3</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>116 ± 3</td>
<td>121 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>71 ± 2</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Upright</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>90 ± 4</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>112 ± 4</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>72 ± 2</td>
<td>71 ± 3</td>
</tr>
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</table>

Values are means ± SE. Upright, after 10 min at 60° head-up tilt; BP, blood pressure; P values are given for the comparison between men and women. Symbols (*, †, ‡) indicate significant differences. NS, not significant.
pressor testing increased SBP by only 4 ± 2 and 9 ± 2 mmHg in women and in men, respectively (NS).

Heart rate variability, blood pressure variability, and baroreflex sensitivity. With placebo, heart rate variability was and baroreflex sensitivity tended to be higher in men (Table 2). NET inhibition attenuated these gender differences. Low-frequency oscillations of SBP with placebo were similar in both genders. NET inhibition markedly decreased low-frequency blood pressure oscillations regardless of gender.

Plasma catecholamines. With placebo, plasma norepinephrine concentrations in the supine position tended to be increased in women compared with men (Table 3). Plasma DHPG concentrations and the DHPG/norepinephrine ratio were not significantly different. Reboxetine caused a similar reduction in supine norepinephrine in both groups (22% in women, 18% in men). In contrast, plasma norepinephrine in the upright position increased with reboxetine (35% in men and 33% in women). Reboxetine led to a marked reduction in plasma DHPG concentrations and the DHPG/norepinephrine ratio in both groups.

Adrenergic Regulation in Abdominal Adipose Tissue

Changes in ethanol ratio and dialysate glycerol with incremental concentrations of tyramine and isoproterenol are given in Fig. 3. The change in the ethanol ratio with either tyramine or isoproterenol was similar in men and in women. The lipolytic response to both agents was augmented in women (P < 0.01 by ANOVA for both interventions). Interstitial glycerol at baseline was 83 ± 16 μM in men and 93 ± 16 μM in women. During stimulation with 1 μM isoproterenol, dialysate glycerol was 163 ± 27 μM in men and 244 ± 19 μM in women (P < 0.01). During stimulation with 0.35 mM tyramine, glycerol was 119 ± 28 μM in men and 216 ± 35 μM in women (P < 0.05). We plotted glycerol responses to 1 μM isoproterenol and 0.35 mM tyramine against each other and found a significant linear relationship (Fig. 4). However, data for men and for women were on the same regression line.

DISCUSSION

We used a combination of pharmacological techniques to obtain insight into NET function in women and in men, both systemically and at the adipose tissue level. First, we studied placebo day, NS). In contrast, reboxetine increased supine cardiac output 14 ± 7% in men (P < 0.05). Systemic vascular resistance did not change significantly in either group.

Hemodynamic response to head-up tilt. With placebo, the tilt-induced increase in heart rate was not influenced by gender (Fig. 1). SBP was well maintained during head-up tilt in both sexes (Table 1). In contrast, women had lower increases in mean and diastolic arterial pressure during head-up tilt than men (Table 1). With head-up tilt, women had less of a decrease in stroke volume (−36 ± 3% compared with −47 ± 3% in men, P < 0.001). With NET inhibition, upright heart rate increased dramatically. The response was more pronounced in men (+56 ± 5 beats/min compared with +42 ± 4 beats/min in women, P < 0.001). With NET inhibition, systolic BP decreased during head-up tilt. Again, the effect was more pronounced in men than women (−16 ± 5 mmHg compared with −5 ± 4 mmHg in women, P < 0.01). NET inhibition had no effect on diastolic blood pressure during tilt testing. The decrease in stroke volume with head-up tilt was augmented with NET inhibition (−62 ± 2% in men, −39 ± 3% in women, P < 0.01).

Autonomic reflex testing. With placebo, handgrip testing increased SBP 25 ± 5 mmHg in women and 24 ± 4 mmHg in men (NS). During NET inhibition, handgrip testing elicted a much smaller pressor response in both groups (9 ± 5 mmHg in women, 13 ± 4 mmHg in men, NS). With placebo, cold pressor testing increased SBP 22 ± 3 mmHg in women and 27 ± 3 mmHg in men (NS). During NET inhibition, cold pressor testing increased SBP by only 4 ± 2 and 9 ± 2 mmHg in women and in men, respectively (NS).

Table 2. Supine heart rate variability, blood pressure variability, and baroreflex sensitivity

<table>
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<tr>
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<th>Placebo</th>
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<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Rmssd, ms</td>
<td>54±13</td>
<td>107±19</td>
</tr>
<tr>
<td>Pm50, %</td>
<td>17±6</td>
<td>33±5</td>
</tr>
<tr>
<td>HF-RRI, ms²</td>
<td>440±130</td>
<td>830±170</td>
</tr>
<tr>
<td>LF-RRI, ms²</td>
<td>700±220</td>
<td>1,450±520</td>
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<tr>
<td>LF/HF-RRI</td>
<td>3±1</td>
<td>2±1</td>
</tr>
<tr>
<td>LF-SBP, mmHg²</td>
<td>9±3</td>
<td>10±2</td>
</tr>
<tr>
<td>BRS-LF, mmHg²</td>
<td>10±2</td>
<td>17±3</td>
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Rmssd, square root of the mean squared differences of successive normal-to-normal intervals; Pm50, proportion of successive normal-to-normal intervals differences greater than 50 ms; HF-RRI, RR variability in the high-frequency range; LF-RRI, RR variability in the low-frequency range; LF/HF-RRI, ratio between RR variability in the low- and the high-frequency range; LF-SBP, systolic blood pressure variability in the low-frequency range; BRS-LF, baroreflex slope in the low-frequency range; BRS+ – baroreflex slope (upsloping segments); BRS–, baroreflex slope (downdoping segments). P values are given for the comparison between men and women. *Significant difference.

Fig. 2. Individual data on the systolic pressor effect of norepinephrine transporter inhibition in the supine position. ΔSBP: change in systolic blood pressure. ◊, Women; □, men. *P < 0.05 by unpaired t-test.
the effect of the selective NET inhibitor reboxetine on cardiovascular regulation and venous catecholamine concentrations. Reboxetine is a highly selective norepinephrine uptake inhibitor and does not bind to muscarinic cholinergic receptors or adrenoreceptors (33). These features make reboxetine a useful pharmacological tool to study NET physiology. Even though we did not adjust the dose for body weight, reboxetine plasma concentrations were similar in men and in women. Sufficient NET inhibition in both groups is suggested by a pronounced reduction in the DHPG/norepinephrine ratio (11, 12).

The heart is particularly dependent on NET function for the removal of norepinephrine from the synaptic cleft (9, 11). Therefore, we expected that a gender difference in the effect of NET inhibition should be particularly evident with respect to cardiac autonomic regulation. NET inhibition attenuated the gender difference in heart rate regulation. With placebo, we observed a marked gender difference in the autonomic regulation of the heart. For example, resting heart rate was reduced and heart rate variability was markedly increased in men compared with women. A lower resting heart rate in men than women has been described in numerous studies (1, 10, 24). With NET inhibition, men exhibited a similar increase in resting heart rate but a more pronounced reduction in heart rate variability compared with women. NET inhibition resulted in a greater increase in upright heart rate in men than women. Men and women had virtually identical heart rate values at 75° head-up tilt after reboxetine. We also observed a gender-related effect of NET inhibition on cardiac stroke volume. In men, cardiac stroke volume increased with NET inhibition, which resulted in increased cardiac output. The increase in cardiac output contributed to the markedly greater pressor effect of reboxetine in men than women. These responses are probably explained by an increase in cardiac norepinephrine spillover with NET inhibition (9).

![Fig. 3. Response to incremental concentrations of isoproterenol and tyramine on tissue blood flow and lipolysis. Tyramine and isoproterenol were administered through microdialysis catheters in subcutaneous abdominal adipose tissue. Blood flow changes were assessed with the ethanol dilution technique. A decrease in the ethanol ratio indicates an increase in tissue blood flow. Dialysate glycerol concentrations were determined as an indicator of lipolysis.](http://ajpregu.physiology.org/ by 10.220.33.6 on June 29, 2017)

Table 3.  

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<th>Placebo</th>
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<th>Reboxetine</th>
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<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>P</td>
<td>Women</td>
<td>Men</td>
<td>P</td>
</tr>
<tr>
<td>Supine DHPG, nmol/l</td>
<td>5.6±0.5</td>
<td>5.1±0.4</td>
<td>3.2±0.2</td>
<td>3.7±0.2</td>
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<tr>
<td>Norepinephrine, nmol/l</td>
<td>1.6±0.3</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.0±0.1</td>
<td>&lt;0.05*</td>
<td></td>
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<tr>
<td>DHPG/norepinephrine</td>
<td>4.1±0.4</td>
<td>4.8±0.5</td>
<td>2.8±0.2</td>
<td>3.9±0.4</td>
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<tr>
<td>Epinephrine, nmol/l</td>
<td>0.06±0.02</td>
<td>0.08±0.03</td>
<td>0.11±0.01</td>
<td>0.08±0.01</td>
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<td>DOPA, nmol/l</td>
<td>9.2±0.76</td>
<td>7.6±0.35</td>
<td>9.1±0.66</td>
<td>8.4±0.71</td>
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<tr>
<td>Upright DHPG, nmol/l</td>
<td>6.5±0.6</td>
<td>6.4±0.5</td>
<td>3.8±0.2</td>
<td>4.2±0.3</td>
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<tr>
<td>Norepinephrine, nmol/l</td>
<td>2.3±0.3</td>
<td>2.6±0.1</td>
<td>3.1±0.2</td>
<td>3.5±0.2</td>
<td></td>
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</tr>
<tr>
<td>DHPG/norepinephrine</td>
<td>3.4±0.6</td>
<td>2.5±0.2</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
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<tr>
<td>Epinephrine, nmol/l</td>
<td>0.7±0.1</td>
<td>0.4±0.1</td>
<td>0.2±0.04</td>
<td>0.3±0.09</td>
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<tr>
<td>DOPA, nmol/l</td>
<td>8.6±0.71</td>
<td>8.1±0.46</td>
<td>8.8±0.71</td>
<td>8.5±0.56</td>
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Upright, after 10 min at 60° head-up tilt; DHPG, dihydroxyphenylglycol; DHPG/norepinephrine, ratio between DHPG and norepinephrine; DOPA, dihydroxyphenylalanine; NET, norepinephrine transporter. Conversion factors to convert to conventional units (pg/ml): 170.07 for DHPG, 169.20 for norepinephrine, 183.15 for epinephrine, and 197.24 for DOPA. P values are given for the comparison between men and women. *Significant difference.
mediated through activation of central nervous system mechanisms, that the sympatholytic effect of NET inhibition in the brain is not preserved by a sympatholytic effect in the brain. Animal studies suggest that an increase in the response to cold-pressor and handgrip testing operations. Systemic vascular resistance remained stable. We did not observe a major gender difference in the effect of NET inhibition on the regulation of systolic blood pressure. We used the microdialysis technique to obtain another measure of peripheral NET function elsewhere in the body. We applied direct and indirect adrenergic agonists locally to adipose tissue, namely isoproterenol and tyramine. Isoproterenol increases lipolysis directly through stimulation of postsynaptic β-adrenoceptors on adipocytes. Tyramine is taken up through NET and releases norepinephrine from postganglionic adrenergic neurons (19). The released norepinephrine acts on postsynaptic receptors on adipocytes. We observed a marked gender difference in the lipolytic response to tyramine. However, we observed a similar gender difference in isoproterenol responses. Thus the gender difference in tyramine responsiveness is probably explained by a difference in postsynaptic adrenergic sensitivity rather than a systemic difference in NET function. One possible conclusion is that NET function in peripheral tissues is not influenced by gender. Another explanation is that a gender difference in NET function is not evenly distributed throughout the body. We favor the explanation that a gender difference in peripheral NET function is phenotypically expressed in organs that are dependent on NET for the removal of norepinephrine from the synaptic cleft. Indeed, our data on heart rate and stroke volume regulation during NET inhibition suggest that the difference is particularly evident at the level of the heart.

Output did not increase with NET inhibition in women. Blood pressure remained stable. We did not observe a major gender difference in the effect of NET inhibition on the regulation of systemic vascular resistance.

We used the microdialysis technique to obtain another measure of peripheral NET function elsewhere in the body. We applied direct and indirect adrenergic agonists locally to adipose tissue, namely isoproterenol and tyramine. Isoproterenol increases lipolysis directly through stimulation of postsynaptic β-adrenoceptors on adipocytes. Tyramine is taken up through NET and releases norepinephrine from postganglionic adrenergic neurons (19). The released norepinephrine acts on postsynaptic receptors on adipocytes. We observed a marked gender difference in the lipolytic response to tyramine. However, we observed a similar gender difference in isoproterenol responses. Thus the gender difference in tyramine responsiveness is probably explained by a difference in postsynaptic adrenergic sensitivity rather than a systemic difference in NET function. One possible conclusion is that NET function in peripheral tissues is not influenced by gender. Another explanation is that a gender difference in NET function is not evenly distributed throughout the body. We favor the explanation that a gender difference in peripheral NET function is phenotypically expressed in organs that are dependent on NET for the removal of norepinephrine from the synaptic cleft. Indeed, our data on heart rate and stroke volume regulation during NET inhibition suggest that the difference is particularly evident at the level of the heart.

Not all the effects of systemic NET inhibition can be explained by decreased norepinephrine uptake in peripheral tissues and subsequent stimulation of postsynaptic adrenoceptors. For example, we observed a reduction rather than an increase in the response to cold-pressor and handgrip testing (23, 26, 32). This paradoxical response is probably explained by a sympatholytic effect in the brain. Animal studies suggest that a sympatholytic effect of NET inhibition in the brain is mediated through activation of central nervous α2-adrenoceptors (8). In humans, systemic NET inhibition is associated with a profound reduction in muscle sympathetic nerve activity (9, 32). Furthermore, systemic as well as renal and forearm norepinephrine spillover decrease with NET inhibition (9). We recently observed a marked reduction in low-frequency oscillations of SBP with NET inhibition (3, 26, 32). Low-frequency oscillations of SBP, the so-called Mayer waves, are mediated by the sympathetic nervous system (25). We observed a similar suppression of SBP low-frequency oscillations in men and women. Moreover, NET inhibition attenuated the cold pressor and handgrip response similarly in men and in women. Finally, NET inhibition reduced supine plasma norepinephrine concentrations similarly in men and in women. These observations might suggest a lesser contribution of central nervous NET to gender differences in cardiovascular regulation compared with NET in peripheral tissues.

We cannot completely exclude the possibility that the gender difference in the response to NET inhibition is explained by factors other than NET activity. A similar reduction in NET function with reboxetine in both genders might lead to different compensatory neural adjustments. Indeed, factors that influence blood pressure control and that have been shown to differ between men and women include vasoactive hormones, blood volume, receptor sensitivity, and distribution, and other autonomic nervous system mechanisms (6, 16, 17, 30). Interestingly, in a family with genetic NET dysfunction, two women reported severe symptoms of orthostatic intolerance (28). Even if the gender difference were explained by a difference in counterregulation rather than a difference in NET function, the contribution of NET to cardiovascular regulation is clearly different.

**Perspectives**

Our study suggests that the contribution of NET to cardiovascular regulation differs between men and women. This gender difference mainly involves cardiac regulation and may explain in part the much greater propensity of women to experience orthostatic tachycardia compared with men. Our data also suggest that men may be more likely to experience a pressor response during treatment with NET inhibitors, at least at the onset of the therapy. However, the mechanisms that contribute to the gender difference are not known. In animal studies, sex hormones influenced the expression and function of NET (27, 34). Therefore, we suspect that the gender difference in sensitivity to NET inhibition in humans might be mediated by sex hormones. We did not measure sex hormone concentrations in the present study. Another potential limitation of our study is that in the reboxetine substudy, women were studied during different stages of the menstrual cycle, and many were using oral contraceptives. We speculate that changes in circulating sex hormones may contribute to changes in adrenergic regulation during the menstrual cycle (17). Given the importance of NET for norepinephrine homeostasis, the paucity of data on the regulation of NET in humans is surprising. We propose that the effect of sex hormones on human NET expression and function should be further characterized.

**ACKNOWLEDGMENTS**

We thank S. Lonce for carrying out the assays of plasma levels of catechols in this study.

**GRANTS**

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