Influences of Norepinephrine Transporter Function on the Distribution of Sympathetic Activity in Humans

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Abstract—Previous studies suggest that neuronal norepinephrine transporter function may regulate the distribution of sympathetic activity among blood vessels, heart, and kidney; we tested the functional relevance in humans. Sixteen healthy men (26±1 years) ingested 8 mg of the selective norepinephrine reuptake transporter inhibitor reboxetine or a matching placebo on 2 separate days in a double-blind, randomized, crossover fashion. We monitored heart rate, thoracic bioimpedance, blood pressure, glomerular filtration rate, and renal blood flow. Ninety minutes after ingestion of the test medication, subjects were tilted to a 45° head-up position, where they remained for an additional 30 minutes. Reboxetine increased supine systolic blood pressure through an increase in cardiac output whereas systemic vascular resistance decreased. Furthermore, reboxetine increased heart rate, particularly with a head-up tilt. Supine plasma renin activity was 0.71±0.15 ng angiotensin (Ang)/L per mL/h with placebo and 0.36±0.07 ng/Ang/L per mL/h with reboxetine (P<0.01). Supine plasma Ang II concentrations were also decreased with reboxetine. Both plasma renin activity and Ang II concentrations remained suppressed during head-up tilt. On placebo, renal vascular resistance increased with head-up tilt. The response was abolished with norepinephrine reuptake inhibition. We conclude that norepinephrine reuptake function profoundly influences the distribution of sympathetic activity between the heart, vasculature, and kidney in humans. All of these changes are physiologically relevant because they lead to corresponding changes in organ function. (Hypertension. 2006;48:120-126.)

Key Words: sympathetic nervous system ■ norepinephrine ■ renal circulation ■ renin-angiotensin system

Sympathetic activity is not evenly distributed among the blood vessels, heart, and kidney. Discordant sympathetic activation of the heart and the kidney may be relevant to common cardiovascular disorders. For example, the postural tachycardia syndrome (POTS) is associated with excessive cardiac sympathetic activity.1 Decreased renal sympathetic activity in POTS is suggested by hypovolemia together with inappropriately low renin–angiotensin system activity.2 In contrast, in obese subjects, renal norepinephrine spillover is increased.3 Sympathetic traffic to the vasculature may also be increased, at least in a subgroup of obese patients.4,5 Cardiac sympathetic activity is suppressed in obese normotensive persons and is within the normal range in obese hypertensive patients.6 Yet, the mechanisms regulating sympathetic distribution between the heart and kidney are poorly understood. Norepinephrine reuptake transporter (NET) function may be important in this regard. Acute pharmacological NET blockade reduces sympathetic vasomotor tone7 and renal norepinephrine spillover in humans.8 In contrast, cardiac norepinephrine spillover5 and upright heart rate are substantially increased.9 Similarly, upright heart rate is increased in patients with genetic NET deficiency.10 Nevertheless, how changes in sympathetic distribution influence the subtle interplay between vascular, cardiac, and renal function is unknown. In particular, how these alterations respond to a change in sympathetic nervous system activity remains unclear. To address these issues, we monitored cardiovascular and renal regulation in healthy subjects in the presence and in the absence of selective NET inhibition, both in the supine position and during head-up tilt.

Methods

Subjects
We studied 16 healthy men (26±1 years, body mass index 24.0±0.5 kg/m²). They received no regular medication. We obtained a standardized medical history, physical examination, and determined routine laboratory tests. The local ethics committee approved the study. Written informed consent was obtained from all subjects before study entry. Experiments were conducted in accordance with national and international regulations and institutional guidelines.

Protocol
We conducted all experiments in the morning hours after an overnight fast. Subjects abstained from alcohol, nicotine, and caf-
feine 48 hours before the experiments. They were tested with 8 mg reboxetine (Edronax, Pharmacia Upjohn) or a matching placebo on 2 separate days in a double-blind, randomized, crossover fashion. The washout phase between tests with reboxetine and with placebo was at least 1 week.

On the testing day, subjects were placed supine on a motorized tilt table. We recorded ECG (Viridia CMS, Hewlett-Packard) and thoracic bioimpedance continuously (Niccomo, Medis GmbH). Brachial blood pressure was measured at regular intervals with an automated cuff (Dinamap, Critikon). Furthermore, blood pressure was measured continuously by a servo-plethysmomanometer on the finger that was kept at heart level throughout the experiments (Finapress 2300, Ohmeda). We inserted 2 venous catheters in contralateral antecubital veins, one catheter for blood sampling and the other for infusion of inulin (Fresenius Kabi) and para-aminohippuric acid ([PAH] Clinalfa).

Infusion and PAH were administered as continuous infusions. After 15 minutes at loading doses, infusion rates for inulin and PAH were reduced to maintenance dosages. Infusion rates for loading doses were calculated as body weight (kg)$\times$0.78$\times$88 mg/h for PAH, respectively. We calculated the estimated glomerular filtration rate (GFR) according to the following formula: $(140-\text{age}[\text{yr}])\times$ weight (kg)/ creatinine (mg/dL)/72. Infusion rates for maintenance dosages were calculated as (estimated GFR)$\times$0.06$\times$200 mg/h for inulin and (estimated GFR)$\times$0.075$\times$88 mg/h for PAH, respectively. After 2 hours of continuous infusion, we achieved steady-state plasma concentrations for both inulin and PAH.

![Figure 1](image-url). Systolic and diastolic blood pressure, heart rate, and changes in stroke volume, cardiac output, and total peripheral resistance in the supine position are shown after ingestion of 8 mg reboxetine (●, drawn line) or placebo (○, dotted line). n=16, *$P<0.05$; **$P<0.01$; ***$P<0.001$ (paired $t$ test).
After steady-state PAH and inulin concentrations were reached, we obtained our baseline measurements. Then, subjects ingested 8 mg reboxetine or matching placebo with 50 mL water. We continued the measurements for another 90 minutes in the supine position and obtained venous blood samples 30, 60, and 90 minutes after drug ingestion. After this period, subjects were tilted to a 45° head-up tilt where they remained for 30 minutes. Blood samples were taken after 25 and 30 minutes at a 45° head-up tilt.

**Data Acquisition and Analysis**

Signals of ECG and thoracic impedance were analog-to-digital converted at 500 Hz using DI-720USB and Windaq Pro+ software (DATAQ Instruments). Data analysis was done offline using a customized waveform analysis software based on PV-Wave (Visual Numerics Inc). Stroke volume and cardiac output were calculated according to Sramek’s formula.11

**Laboratory Measurements**

Reboxetine plasma concentrations were measured with high-performance liquid chromatography (HPLC) as described previously. For hematocrit determination, glass capillaries were filled with EDTA blood, centrifuged for 15 minutes at 3000 rpm, and read out with a nomogram. For plasma renin activity, angiotensin II, and aldosterone measurements, blood samples were drawn into pre-chilled tubes, immediately centrifuged, stored at −18°C, and then analyzed as described previously. Plasma catecholamines were determined by a modification of an HPLC method. PAH and inulin were measured as described earlier.13

**Calculations and Statistics**

Renal plasma flow (RPF) was calculated as the quotient of PAH infusion rate and PAH plasma concentration. Renal blood flow (RBF) was computed as RPF/(100−hematocrit)×100. Renal vascular resistance was computed as the quotient of mean brachial blood pressure and RBF. GFR was computed as the quotient of inulin infusion rate and inulin plasma concentration. Filtration fraction was calculated as (quotient of GFR and RPF)×100.

All data are expressed as mean±SEM. Intraindividual and inter-individual differences were compared by paired and unpaired t tests, respectively. ANOVA testing for repeated measures was used for multiple comparisons. P<0.05 was considered significant.

**Results**

**Measurements in the Supine Position**

Venous reboxetine plasma concentrations were 135±11 ng/mL 90 minutes after reboxetine ingestion and 0±0 ng/mL after placebo ingestion. Hemodynamic data in the supine position with placebo and with reboxetine are illustrated in Figure 2.

**Figure 2. RBF and renal vascular resistance in the supine position after ingestion of 8 mg reboxetine (●, drawn line) or placebo (○, dotted line). n=16, *P<0.05 (paired t test).**

**Figure 1. Reboxetine increased systolic blood pressure from 118±3 mm Hg at baseline to 133±3 mm Hg 90 minutes after drug ingestion (P<0.01). Diastolic blood pressure did not change. Reboxetine increased supine heart rate from 60±3 to 69±2 bpm (P<0.05). The pressor response to reboxetine was driven by a 19±4% (P<0.001) increase in cardiac output. In contrast, systemic vascular resistance decreased 8±2% with reboxetine (P<0.001). None of these measurements changed with placebo.**

With reboxetine, supine RBF increased from 1270±42 mL/min at baseline to 1350±48 mL/min 90 minutes after drug ingestion (Figure 2, P<0.05). Supine renal vascular resistance did not change with reboxetine. Supine RBF and renal vascular resistance remained stable with placebo. Reboxetine and placebo had no effect on GFR or filtration fraction in the supine position.

Changes in supine renin–angiotensin–aldosterone system activity with reboxetine and with placebo are illustrated in Figure 3. Reboxetine markedly attenuated supine plasma renin activity from 0.71±0.15 to 0.36±0.07 ng angiotensin/L per mL/h (P<0.01). Supine plasma angiotensin II concentrations decreased from 5.6±0.7 to 3.2±0.3 pg/mL (P<0.01). Supine aldosterone concentrations did not change. All these hormonal measurements did not change with placebo. Supine plasma norepinephrine or epinephrine concentrations did not change significantly with either reboxetine or placebo.

**Response to Head-Up Tilt**

Head-up tilt testing had to be aborted prematurely in 6 subjects on the placebo day and in 1 subject on the reboxetine day because of symptomatic presyncope. The complete data set in the supine and in the upright position was available in 10 subjects. Hemodynamic responses to head-up tilt testing in these subjects are illustrated in Figure 4. Head-up tilt increased heart rate 22±4 bpm with placebo and 38±6 bpm with reboxetine (P<0.01). Systolic and diastolic blood pressure was well maintained with placebo. Systolic blood pressure decreased 14±5 mm Hg with reboxetine (P<0.02) whereas diastolic blood pressure remained stable. Thus, upright blood pressure was similar with placebo and with reboxetine. With the head-up tilt, cardiac output decreased to the same extent in both treatment groups (−14±5% with placebo, −10±5%
change during head-up tilt (27±41 mL/min). Moreover, renal vascular resistance did not change during head-up tilt testing (0±5%). Thus, reboxetine attenuated renal vasoconstriction with upright posture. With reboxetine, GFR and filtration fraction did not change during the tilt test.

With placebo and with reboxetine, plasma renin activity, angiotensin II, and aldosterone increased during head-up tilt (Figure 5). However, plasma renin activity and angiotensin II were lower with reboxetine compared with placebo. Upright aldosterone concentrations were similar with both interventions. Furthermore, there was no difference in upright epinephrine and norepinephrine concentrations with placebo and with reboxetine.

Discussion

We used the selective NET inhibitor reboxetine as a pharmacological tool to study the role of NET function in cardiovascular and renal regulation. In the doses applied here, reboxetine does not inhibit dopamine or serotonin uptake. Furthermore, reboxetine does not interfere with adrenergic or cholinergic receptors. Thus, all the responses to reboxetine are related to norepinephrine uptake inhibition rather than a nonspecific effect of the drug. The novel finding of our study is that NET inhibition substantially alters the interplay between cardiovascular and renal regulation, both in the supine and in the upright position. For example, cardiac output was increased during NET inhibition, whereas the activity of the renin–angiotensin system was profoundly suppressed. Furthermore, during NET inhibition, the heart rate response to orthostatic stress was augmented. In contrast, renal vascular resistance failed to increase properly with upright posture.

The paradoxical findings in our study are probably explained by the complex effect of NET inhibition on the sympathetic nervous system. With an exclusively peripheral site of action one would expect NET inhibitors to increase synaptic norepinephrine concentrations, which in turn should increase sympathetic responses. Systemic NET inhibition elicits a more complicated response, presumably through interaction of peripheral and central nervous responses. When NET inhibitors are applied directly to the brain, sympathetic activity decreases substantially. The sympatholytic response is likely to be mediated through central nervous system adrenoceptors. Also with systemic administration, NET inhibition elicits a central sympatholytic response in rabbits and humans. The overall response to NET inhibition in each organ depends on the balance between peripheral stimulatory and central nervous inhibitory mechanisms. NET inhibition with desipramine reduces renal and forearm norepinephrine spillover, but increases cardiac norepinephrine spillover in healthy subjects.

One possible explanation for the discrepant sympathetic response between organs is an anatomic difference in adrenergic synapses. In the heart, pre- and postsynaptic membranes are located particularly close to each other. This anatomic feature makes the heart more dependent on NET for removal of norepinephrine from the synaptic cleft. Indeed, a larger proportion of the released norepinephrine is taken up through NET in the heart compared with other organs. Another possible explanation for the discordant effect of NET inhibi-
tion on the heart, vasculature, and kidney is an organ-specific alteration in baroreflex regulation. In an earlier study, NET inhibition with reboxetine was associated with a shift in the baroreflex heart rate curves such that at a given blood pressure heart rate was increased. In contrast, baroreflex regulation of sympathetic vasomotor tone was attenuated. Redistribution of baroreflex outflow from the vasculature toward the heart has also been described in patients with POTS; NET inhibition could have a similar effect on baroreflex regulation of renal sympathetic nerve activity.

If the organ-specific changes in norepinephrine turnover with NET inhibition were physiologically relevant, one would expect to see corresponding changes in sympathetic responses in each affected organ, particularly during sympathetic stimulation. Cardiac sympathetic activity regulates heart rate and cardiac contractility. Given the increase in cardiac norepinephrine spillover with NET inhibition, heart rate and cardiac output are expected to increase. In any event, heart rate and cardiac output increased with NET inhibition in our study. Similarly, selective and nonselective NET inhibition increased heart rate in the supine position and even more so in the upright position. Similar abnormalities in heart rate regulation have been described in patients with familial NET deficiency. The pressor response to nonselective NET inhibition can be prevented with β-adrenoreceptor blockade. Clearly, changes in cardiac norepinephrine turnover with NET inhibition are functionally relevant.

Considering the norepinephrine spillover data, vascular and renal sympathetic responses should be decreased during NET inhibition. Vascular sympathetic efferents control vascular tone. In the present study, systemic vascular resistance decreased during NET inhibition. Furthermore, selective and nonselective NET inhibition attenuated the response to cold pressor testing in several previous studies. Thus, the reductions in sympathetic vasomotor tone and norepinephrine spillover are indeed associated with attenuated vascular sympathetic responses.
Renal sympathetic responses are more diverse and therefore are particularly difficult to assess and to interpret in humans. The kidney is densely innervated by autonomic, predominantly adrenergic, efferent neurons. In animals, electrical stimulation of renal nerves leads to a graded increase in renin–angiotensin system activity, sodium reabsorption, and renal vascular resistance, depending on stimulation frequency. For each response, sympathetic activity must exceed a certain threshold. Moderate increases in renal sympathetic nerve activity can directly reduce sodium excretion and activate the renin–angiotensin system without major changes in renal blood flow and glomerular filtration. Sympathetically mediated sodium reabsorption is explained in part by direct activation of renal tubular \( \alpha \)-adrenoreceptors. The response is attenuated with phenoxybenzamine and mimicked by norepinephrine. The threshold for renal hemodynamic changes during sympathetic stimulation is higher than the threshold for hormonal responses or for tubular sodium reabsorption. In dogs, a 5-fold increase in renal nerve activity was required to decrease RBF by 40%.

In our study, we assessed renin–angiotensin system activity as a low threshold sympathetic response and renal vascular resistance as a higher threshold response. In the supine position, sympathetic activity is relatively low. Thus, a further reduction in renal nerve activity is likely to exert an effect on sodium excretion and the renin–angiotensin system only, whereas renal perfusion or GFR are unlikely to change to a significant degree. Indeed, in the supine position, we observed changes in renin–angiotensin system activity but no change in renal hemodynamics. In the upright position, sympathetic activity may be sufficient to elicit renal vasconstriction. Thus, inhibition of renal sympathetic activity with NET inhibition could only elicit a hemodynamic response during orthostatic stress.

Our study has several potential limitations. We used only one reboxetine dose in our study. The distribution of sympathetic activity between organs may be related to the degree of NET inhibition. The degree of NET inhibition may also influence the balance between central sympathetic inhibition and peripheral sympathetic stimulation. In contrast to previous studies, upright norepinephrine concentrations were not increased in this study. Higher reboxetine plasma concentrations and higher tilt angles in previous studies may account for this discrepancy. We only studied men. However, NET inhibition has a gender-specific effect on the cardiovascular system. Thus, our findings cannot be simply extrapolated to women. Finally, we cannot exclude completely that reboxetine directly interfered with some of our laboratory measurements. Despite these issues, our study strongly suggests an important effect of NET on the distribution of sympathetic activity between the heart, the vasculature, and the kidney.

Figure 5. Plasma renin activity (upper panel) and plasma concentrations of angiotensin II (middle panel) and aldosterone (lower panel) in the supine position and after 30 minutes of 45° head-up tilt (HUT) with placebo and reboxetine. **P<0.01, #P<0.05 (paired t test).

Perspectives

Our study supports the idea that NET function profoundly influences the distribution of sympathetic activity between the heart, vasculature, and kidney. All of these changes are physiologically relevant because they lead to corresponding changes in organ function. Thus far, no other target has been shown to have a stronger effect on sympathetic distribution than NET. Our findings may be clinically relevant given the widespread clinical use of NET inhibitors. Furthermore, genetic variability in NET function may not only occur in the rare patient with NET deficiency, but also in the general population. Several single nucleotide polymorphisms in the NET gene are associated with substantial alterations in NET function. Moreover, abnormalities in the distribution of sympathetic activity between the heart, vasculature, and kidney have been described with aging, as in different pathophysiological conditions, such as heart failure, POTS, and obesity. NET abnormalities may possibly be important in this regard.

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Disclosures
None.

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