Selective Impairment in Sympathetic Vasomotor Control With Norepinephrine Transporter Inhibition

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Background—Norepinephrine transporter (NET) inhibition increases the responsiveness to vasoactive medications and attenuates the response to sympathetic stimuli. The phenomenon may be a result of impaired regulation of sympathetic vasomotor tone.

Methods and Results—We studied the effects of the selective NET blocker reboxetine and placebo on baroreflex control of heart rate (HR) and sympathetic traffic in a randomized, double-blind, crossover manner in healthy subjects. Subjects ingested 8 mg reboxetine or placebo 12 hours and 1 hour before testing. ECGs were measured for HR, brachial and finger blood pressure (BP), and muscle sympathetic nerve activity (MSNA). Sympathetic and parasympathetic baroreflex slopes were determined by use of incremental phenylephrine and nitroprusside infusions. The dose to reach BP changes of 12.5 mm Hg was significantly lower during NET inhibition (0.25 versus 0.64 μg · kg⁻¹ · min⁻¹ phenylephrine and 0.40 versus 1.10 μg · kg⁻¹ · min⁻¹ nitroprusside, P<0.01). Baroreflex control of HR was similar (16 ms/mm Hg with placebo versus 14 ms/mm Hg with reboxetine) but reset to higher BP values. MSNA and sympathetically mediated low-frequency BP oscillations were profoundly reduced at baseline and failed to increase sufficiently during nitroprusside infusion. Reboxetine attenuated BP and MSNA responses to cold pressor testing.

Conclusions—NET inhibition profoundly and selectively reduces baroreflex control of sympathetic vasomotor tone and attenuates the responsiveness to sympathetic stimuli. The reduction in baroreflex buffering increases the sensitivity to vasoactive medications. Therefore, our findings represent a novel mechanism for drug interactions. (Circulation. 2003;107:2949-2954.)

Key Words: nervous system, autonomic ■ baroreflex ■ antidepressive agents ■ receptors, adrenergic, alpha ■ catecholamines

Approximately 80% to 90% of released norepinephrine is taken up again by postganglionic adrenergic neurons through the norepinephrine transporter (NET) and repackaged or metabolized. Most antidepressants, sibutramine,1 and illicit drugs, such as cocaine, inhibit NET. Given the importance of norepinephrine homeostasis in cardiovascular and metabolic regulation and the widespread use of NET inhibitors, one would expect a large body of literature on NET physiology. However, the issue has been largely neglected. Clinical studies implicating impaired norepinephrine uptake in the pathogenesis of essential hypertension2 and orthostatic intolerance3,4 renewed interest in NET physiology. A particular impetus was the recent description of a family in which orthostatic intolerance segregated with a functional NET gene mutation.3 When applied to isolated organs or given intra-arterially in low doses, NET inhibitors increase norepinephrine washout.5 Thus, one might expect that a decrease in NET function exerts a profound increase in blood pressure (BP) and heart rate (HR) through increased synaptic norepinephrine. Indeed, NET dysfunction or blockade is associated with marked orthostatic tachycardia and increased upright plasma norepinephrine concentrations.3,4 However, NET inhibition results in a profound decrease in basal sympathetic nerve traffic, low-frequency oscillations in systolic BP (SBP), and systemic norepinephrine spillover.4,5,6 Moreover, the pressor response to sympathetic stimuli is strongly attenuated.4,7 Finally, NET inhibition substantially increases sensitivity to various vasoactive drugs,4 which may be related to impaired baroreflex BP buffering.8 We tested the hypothesis that the paradoxical decrease in sensitivity to sympathetic stimuli and hypersensitivity to vasoactive medications with NET inhibition are explained by a selective impairment in sympathetic vasomotor regulation.

Methods

Subjects
We studied 12 healthy control subjects (11 men, 1 woman; age, 32±6 years; body mass index, 24±3 kg/m²). Written informed consent was obtained from all participants. The study was approved by the local ethics committee.
Three minutes before the application of the next dose were averaged.

Fourier transform algorithm (step, 60 seconds; window, 64 seconds).

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ingested 8 mg reboxetine or matching placebo 12 hours and 1 hour

Acute Effect of NET Inhibition

After instrumentation and recording of a stable baseline for ≥30

minutes, 6 healthy subjects (5 men, 1 woman; age, 36 ± 2 years; body

mass index, 23 ± 2 kg/m²) ingested 8 mg of the selective NET

inhibitor reboxetine (Edronax, Pharmacia Upjohn). Recordings were

continued for an additional 120 minutes. A cold pressor test was

performed by placing 1 hand in ice water (50% ice, 50% water) for

1 minute at baseline and every 30 minutes after reboxetine ingestion.

Blood samples were taken every 30 minutes. Plasma reboxetine

levels were determined by high-performance liquid chromatography

with column-switching and ultraviolet detection.

Crossover Study With NET Inhibition

Ten male subjects (age, 31 ± 6 years; body mass index, 24 ± 4 kg/m²)

ingested 8 mg reboxetine or matching placebo 12 hours and 1 hour

before the study as described previously.9,10 Studies with placebo

and with reboxetine were conducted on separate days in a double-

blind crossover manner after an overnight fast. After a stable

baseline had been reached, subjects underwent cold pressor testing.

Thereafter, incremental infusions of sodium nitroprusside and phen-
ylephrine hydrochloride (0.2, 0.4, 0.8, and 1.6 μg · kg⁻¹ · min⁻¹ over

a period of 5 minutes) were given. The infusions were stopped after

the maximum dose had been given or after diastolic BP had changed

by >15 mm Hg. After baseline values had returned, bolus injections of

propranolol were applied every 3 minutes (0.01, 0.02, 0.04, 0.06,

and 0.08 mg/kg) in incremental doses. Subjects received a total

intravenous 0.21-mg/kg propranolol dose within 15 minutes. Cold

pressor testing was repeated during β-blockade.

Data Acquisition and Analysis

Data were converted from analog to digital at 500 Hz using the

Windaq pro+ software (Dataq Instruments Inc). R-R intervals,
diastolic BP and SBP values, and respiration were defined offline for

the complete records using a program written by one of the authors

(A.D.) that is based on PV-wave software (Visual Numerics Inc).

MSNA bursts were identified after the integrated signal had been

filtered and the baseline defined according to following criteria: (1)

signal-to-noise ratio (set to 2.5), (2) tolerance limits of the skewness

of the rising and falling parts of the bursts, (3) latency limit, (4) burst

width limit (short duration = artifact, long duration = skin sympathetic

nerve activity or afferent activity), and (5) no preceding premature

beats.9

Spectral Analysis and Baroreflex Sensitivity

Pharmacological baroreflex sensitivity was determined from the

steepest part of the sigmoidal curve obtained during phenylephrine

and sodium nitroprusside infusions. HR and BP variability were
determined by use of spectral analysis, cross-spectral analysis, and
the transfer function between R-R intervals and SBP. Analysis was
performed for 5-minute segments during different baselines before
each intervention.11,12 To obtain reliable spectra during phenylephrine
and sodium nitroprusside infusions, we used a sliding fast
Fourier transform algorithm (step, 60 seconds; window, 64 seconds).
Three minutes before the application of the next dose were averaged.

Results

Acute NET Inhibition

Changes in reboxetine plasma concentration over time are illustrated in Figure 1 (top). The maximum of the reboxetine plasma concentration was reached after ~90 minutes. BP was 124 ± 6/72 ± 5 mm Hg at baseline and increased to a maximum of 143 ± 8/81 ± 6 mm Hg 120 minutes after reboxetine ingestion (P < 0.01) (Figure 1, middle). HR increased from
Resting MSNA (normalized area under the burst) was 9.9 ± 2.6 arbitrary units (AU) at baseline. Within 30 minutes of reboxetine ingestion, MSNA started to decrease and reached a minimum of 1.2 ± 0.5 AU after 90 minutes (Figure 1, bottom). The decrease in resting MSNA was associated with a profound reduction in the cold pressor response (Figure 2, top). Moreover, reboxetine substantially reduced the MSNA increase during cold pressor testing (Figure 2, bottom). MSNA recordings were maintained throughout the study in all subjects.

Crossover Study With NET Inhibition

Resting BP and HR

BP was 118 ± 4/66 ± 2 mm Hg with placebo and 133 ± 5/73 ± 3 mm Hg with reboxetine with the subject resting in the supine position (P < 0.01). Supine HR was 60 ± 2 bpm with placebo and 68 ± 2 bpm with reboxetine. As in the acute study, reboxetine attenuated the cold pressor response. During cold pressor testing, BP increased 13 ± 3/12 ± 2 mm Hg with placebo and 6 ± 3/3 ± 2 mm Hg with reboxetine (P < 0.01). Reboxetine also blunted the handgrip response (data not shown).

Basal Sympathetic Nerve Activity

With placebo, MSNA recordings of good quality were obtained in all subjects. Proper positioning of the neurography needle was tested by eliciting afferent nerve activity with tapping on the innervated muscle, by inducing muscle twitching with internal stimulation, provoking MSNA bursts with bolus injections of sodium nitroprusside or during inspiratory breath-hold in all subjects. However, during reboxetine, recordings with spontaneous, pulse-synchronous MSNA bursts were recorded in only 5 subjects. Resting MSNA was reduced profoundly with reboxetine (8.2 ± 2.0 AU with placebo, 1.3 ± 0.2 AU with reboxetine; P < 0.01). Figure 3 illustrates representative BP and MSNA recordings with placebo and reboxetine. In this individual, basal MSNA was reduced profoundly with reboxetine but increased after nitroprusside bolus application.

Responses to Phenylephrine and Nitroprusside

Reboxetine substantially increased sensitivity to phenylephrine and nitroprusside (Figure 4, top). The phenylephrine dose to increase BP 12.5 mm Hg was 0.25 μg · kg⁻¹ · min⁻¹ with reboxetine and 0.64 μg · kg⁻¹ · min⁻¹ with placebo (P < 0.01). The nitroprusside dose that decreased SBP 12.5 mm Hg was 0.40 μg · kg⁻¹ · min⁻¹ with reboxetine and 1.10 μg · kg⁻¹ · min⁻¹ with nitroprusside (P < 0.01). The maximal steepness of the baroreflex HR curves was similar during placebo and during reboxetine (16 ± 2 ms/mm Hg with placebo versus 14 ± 2 ms/mm Hg with reboxetine) (Figure 4, middle). However, the baroreflex curves were reset to higher BP values. The sympathetic baroreflex curve that was obtained in 4 subjects was remarkably distorted with reboxetine (Figure 4, bottom). With placebo, MSNA increased properly with nitroprusside and was suppressed with phenylephrine. With reboxetine, MSNA was very low at baseline and did not decrease further with phenylephrine. Nitroprusside lowered
BP but failed to increase MSNA properly. Only when BP was markedly reduced did MSNA begin to increase.

We determined HR and BP variability at baseline and at each infusion step. The variability data were plotted against BP (Figures 5 and 6). With placebo, HR variability in the high- and low-frequency ranges was reduced as BP was lowered with nitroprusside. Reboxetine reduced HR variability in both frequency ranges independently of BP. However, the ratio between HR variability in the low-frequency and high-frequency ranges was increased with reboxetine. Rebox-
etamine profoundly reduced low-frequency oscillations of SBP even during nitroprusside infusions.

**Response to Propranolol**

Complete β-blockade with propranolol did not result in a significant BP reduction during either placebo or reboxetine. With placebo, R-R interval length was 962 ± 32 ms before and 1060 ± 38 ms with propranolol. With reboxetine, R-R interval length was 878 ± 40 ms before and 1003 ± 35 ms with propranolol. Propranolol had no effect on the HR or BP response to cold pressor testing during placebo or during reboxetine treatment.

**Discussion**

We determined the effect of NET inhibition with reboxetine on the regulation of sympathetic vasomotor control. Reboxetine is a highly selective NET inhibitor and does not bind to muscarinic cholinergic receptors or adrenoceptors. Therefore, reboxetine provides a useful pharmacological tool to selectively manipulate norepinephrine uptake in clinical studies. Reboxetine in the doses used in the present study inhibits norepinephrine uptake sufficiently, as indicated by a robust reduction in the ratio between venous dihydroxypenylglycol and norepinephrine.

We observed a substantial suppression in sympathetic traffic with selective NET inhibition. NET inhibition may influence both the discharge pattern of individual postganglionic sympathetic vasomotor discharge. They increase during sympathetic stimulation. Mayer waves are related to oscillations in sympathetic vasomotor activity. They increase during sympathetic stimulation. In healthy normotensive subjects, BP increases with NET inhibition. Therefore, the decrease in sympathetic vasomotor tone could be caused by baroreflex-mediated inhibition. If the inhibition of sympathetic tone were mediated by the baroreflex, BP lowering would reverse the effect. In any event, MSNA and low-frequency oscillations in SBP remained suppressed during nitroprusside infusion. Thus, the decrease in MSNA is not explained solely by baroreflex-mediated inhibition.

Our findings are consistent with the idea that the decrease in sympathetic vasomotor tone is largely explained by a direct effect on the central nervous system. Stimuli that are normally followed by robust increases in BP, such as handgrip or cold pressor testing, hardly elicit a response during NET inhibition. Our study demonstrates that the phenomenon can be explained in part by decreased responsiveness of sympathetic vasomotor neurons. A reduction in sympathetic activity was also observed in rabbits when desipramine was applied locally to the brain. The reduction in sympathetic activity is mediated at least in part by central nervous system α2-adrenoceptors.

In addition to the effect of selective NET inhibition on sympathetic vasomotor tone, we observed changes in HR regulation. With NET inhibition, the baroreflex HR curve was reset to higher BP values. The shape of the baroreflex curve was maintained. Thus, at a given BP, HR is increased markedly during NET inhibition. The shift of the baroreflex HR curve may contribute to the orthostatic tachycardia observed in patients with NET deficiency and in individuals treated with NET inhibitors. The change in HR regulation was also reflected in the HR variability data. The change in BP with NET inhibition makes the interpretation of HR variability data difficult. Therefore, we plotted HR variability data against BP during different infusion steps with nitroprusside and phenylephrine. NET inhibition resulted in a reduction in HR variability, particularly during nitroprusside infusion. The ratio of low-frequency to high-frequency oscillations of the R-R interval, which is sometimes referred to as sympathovagal balance, was increased during NET inhibition. This finding is supported by studies using the norepinephrine spillover technique. In these studies, NET inhibition was shown to cause disproportional sympathetic stimulation of the heart compared with the vasculature and the kidney. This phenomenon was attributed primarily to the fact that the heart is more dependent on NET to remove norepinephrine from the synaptic cleft. Our study suggests that the oscillatory component of sympathetic HR and vasomotor regulation is also shifted toward the heart. Thus, a central nervous system mechanism may also contribute to the disproportional stimulation of the heart with NET inhibition. A similar mechanism has also been postulated in orthostatic intolerance patients.

We observed a major increase in the sensitivity to phenylephrine and nitroprusside infusions during selective NET inhibition. Similarly, selective NET inhibition increases the sensitivity to phenylephrine and nitroprusside bolus doses. Moreover, isoproterenol infusion exerts a marked depressor response during NET inhibition but not during placebo treatment. The depressor response during phase 2 of the Valsalva maneuver is also increased during NET inhibition. Considering the different modes of action of all these stimuli, it is not likely that the hypersensitivity can be explained entirely by a change in vascular sensitivity. Instead, we suggest that NET inhibition interferes with baroreflex BP buffering. In patients with damage to the afferent baroreflex, the sensitivity to phenylephrine and nitroprusside is increased dramatically. Dysfunction of the efferent baroreflex arc because of either neuronal degeneration (autonomic failure) or ganglionic blockade profoundly increases the sensitivity to vasoactive medications. The baroreflex buffers changes in BP through adjustment of HR and sympathetic vasomotor tone. The hypersensitivity to vasoactive medications during NET inhibition cannot be explained by impaired baroreflex HR control. Close to the operating point, the steepness of the baroreflex HR curves was virtually identical during placebo and during reboxetine. Our study suggests that the decrease in baroreflex BP buffering is explained by a selective increase in baroreflex vasomotor control, because MSNA and low-frequency BP failed to increase properly during nitroprusside infusions. Our findings may explain in part why BP tends to increase with acute NET inhibition even though circulating norepinephrine and whole-body norepinephrine spillover decrease. The same amount of endogenous norepinephrine may elicit a greater pressor response in the setting of impaired baroreflex buffering.
Our findings may have important clinical implications. Pharmacological NET inhibition, which is widely applied in clinical practice, not only suppresses resting sympathetic vasomotor tone but also impairs the ability to respond to sympathetic stimuli. We hypothesize that in conditions associated with high sympathetic vasomotor tone, NET inhibition might lower resting sympathetic activity. Our study also confirms previous studies suggesting that NET is important not only in the regulation of overall sympathetic tone but also in the distribution of sympathetic activity to different organs. NET inhibition selectively attenuates the efficiency of the baroreflex to regulate sympathetic vasomotor tone. This mechanism may contribute to orthostatic hypotension in the elderly. Finally, the selective sympathetic baroreflex dysfunction sensitizes the cardiovascular system to vasoactive medications and represents a novel mechanism for drug interactions.

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