Norepinephrine Transporter–Deficient Mice Exhibit Excessive Tachycardia and Elevated Blood Pressure With Wakefulness and Activity

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Background—Norepinephrine (NE) is a primary neurotransmitter of central autonomic regulation and sympathetic nerve conduction, and the norepinephrine transporter (NET) is crucial in limiting catecholaminergic signaling. NET is sensitive to antidepressants, cocaine, and amphetamine. NET blockade often is associated with cardiovascular side effects, and NET deficiency is linked to tachycardia in familial orthostatic intolerance.

Methods and Results—We telemetrically monitored NET-deficient (NET−/−) mice to determine the cardiovascular effects of reduced NE reuptake. Mean arterial pressure was elevated in resting NET−/− mice compared with NET+/- controls (103±0.6 versus 99±0.4 mm Hg; P<0.01), and corresponding pressures increased to 122±0.3 and 116±0.3 mm Hg (P<0.0001) with activity. Heart rate was also greater in resting NET−/− mice (565±5 versus 551±3 bpm; P<0.05), and genotypic differences were highly significant during the active phase (640±5 versus 607±3 bpm; P<0.0001). Conversely, the respiratory rate of resting NET−/− mice was dramatically reduced, whereas increases after the day/night shift surpassed those of controls. Plasma catecholamines in NET−/− and NET+/- mice were as follows: NE, 69±8 and 32±7; dihydroxyphenylglycol, 2±0.4 and 17±3; epinephrine, 15±3 and 4±0.6; and dopamine, 13±4 and 4±1 pmol/mL. Catechols in urine, brain, and heart also were determined.

Conclusions—Resting mean arterial pressure and heart rate are maintained at nearly normal levels in NET-deficient mice, most likely as a result of increased central sympathoinhibition. However, sympathetic activation with wakefulness and activity apparently overwhelms central modulation, amplifying peripheral catecholaminergic signaling, particularly in the heart. (Circulation. 2004;110:1191-1196.)

Key Words: norepinephrine □ tachycardia □ blood pressure □ nervous system, autonomic □ physiology

The norepinephrine transporter (NET) is critical in central modulation of sympathetic tone and autonomic regulation of peripheral hemodynamics. The amount and type of neurotransmitter removed at any given site vary.1 NET removes not only residual norepinephrine (NE) but, in regions such as the rat prefrontal cortex, a major portion of surplus dopamine as well.2,3 Peripherally, NET is perhaps most important in the heart.4,5 In humans, approximately 92% of cardiac NE removal occurs via NET, with the remainder eliminated by extraneuronal carriers and circulatory dissipation.6 Pharmacological blockade of NET increases extraneuronal concentrations of its substrate(s),7 a mechanism employed in the treatment of depression and anxiety,8,9 although autonomic side effects are common. Systemic administration of desipramine, for example, reduces sympathetic outflow in rodents,10,11 as does the selective NET blocker reboxetine in humans.12,13 Cardiovascular side effects of tricyclic antidepressants include orthostatic hypotension, reduced heart rate (HR) variability, prolonged QT interval, and postural tachycardia,12,14,15 suggesting enhanced peripheral noradrenergic signaling despite increased central sympathoinhibition. Impaired NET function is linked to familial orthostatic intolerance,16 an autonomic disorder characterized by excessive tachycardia, minimal blood pressure changes, and increased NE on assumption of upright posture. A dominant negative mutation in human NET reduces its surface expression via heterologous oligomerization,17 thus rendering a major fraction of the normal transporter inert and reducing NE reuptake in affected heterozygotes.18 Although the gene is completely inactivated in NET-deficient (NET−/−) mice, we hypothesized that the cardiovascular phenotype of NET-deficient humans would be more analogous to that of homozygous, rather than heterozygous, NET-deficient mice.
because of the dominant negative effect of the human polymorphism.

Phenotyping of NET-deficient mice thus far includes decreased depression-like behavior, altered responsiveness to antidepressants, hypersensitivity to psychostimulants, and enhanced sensitivity of the midbrain dopaminergic system. Reported cardiovascular physiology, however, is limited to detection of elevated baseline HR in a test of nociception and echocardiography revealing no structural abnormalities. Therefore, to further characterize the hemodynamic consequences of NET deficiency, we telemetrically monitored conscious NET−/− mice to determine baseline mean arterial pressure (MAP), HR, respiratory rate, and locomotor activity at rest (daytime) and during activity (nighttime) and measured catecholamines in plasma, urine, brain, heart, and adrenal glands.

Methods

Animals

Mice were provided by Dr Marc Caron of Duke University, with NET gene disruption via insertion of enhanced green fluorescent protein CDNA coupled to the PGK-neomycin-resistance gene in exon 2 of the native gene. NET−/− mice derived from an interbred 129SvJ/C57BL/6J strain were backcrossed 10 onto the C57BL/6J strain. NET−/−, NET−/+ and NET+/+ mice were bred and maintained with a 12/12-hour light/dark cycle and fed standard mouse chow with tap water available ad libitum. The Vanderbilt University Institutional Animal Care and Use Committee approved the protocols.

Surgery

Mouse blood pressure radiotometry (Data Sciences International) was described previously. Briefly, mice were anesthetized with 2% to 3% isoflurane, and body temperature was maintained at 36°C to 37°C with an isothermal pad (Braintree Scientific, Inc). After antiseptic preparation, the left carotid artery was isolated, and the lumen was cut to allow insertion of the transmitter catheter to the point of bifurcation. The transmitter body was placed in a lateral subcutaneous pocket. The skin was sutured and secured with adhesive (Nexaband, Veterinary Products Laboratories).

Hemodynamics

NET−/− and NET−/+ mice, aged 3 to 12 months, were monitored individually in their home cage, and beat-to-beat blood pressure data were recorded with the use of DSI ART Gold software. MAP, HR, respiratory rate, and locomotor activity were determined at rest (daytime) and during activity (nighttime). Data were analyzed by customized MatLab software (The Mathworks) in 10-minute increments, averaged over 12-hour time periods, to coincide with the light/dark cycle.

Catecholamines

Catecholamines were measured in plasma from isoflurane-anesthetized mice, from 24-hour urine samples, or from whole-tissue homogenates. Blood was removed via cardiac puncture and preserved with EGTA and reduced glutathione, and organs were harvested immediately thereafter. Urine recepctacles contained 6N HCl to retard catechol breakdown. Catecholamines were determined after alumina extraction by high-performance liquid chromatography with electrochemical detection.

Statistical Analysis

Data are presented as mean±SEM. A 2-way ANOVA with repeated-measures test was used to compare mean hemodynamic values from NET−/− and NET−/+ mice over time, and the rank sum test was used to compare delta values. Catecholamines were analyzed with ANOVA followed by the Dunnett posttest, comparing NET−/− and NET−/+ mouse data with those of NET+/+ mice. Probability <0.05 was considered significant.

Results

Catecholamines

Plasma NE, dopamine (P<0.05), and epinephrine (P<0.01) were significantly increased in NET−/− mice compared with NET−/+ and NET−/+ mice (Figure 1A), whereas the intracellular metabolites dihydroxyphenylglycol (DHPG) (P<0.001) and 3,4-dihydroxyphenylacetic acid (DOPAC; P<0.05) were decreased. Plasma dihydroxyphenylalanine (DOPA) was also lower in NET−/− mice (P=0.052). Urinary catecholamines revealed a similar pattern, with increased NE and decreased DHPG in NET−/− mice (Figure 1B). NET−/+ was significantly decreased in brain and heart (Figure 1C) of NET−/− mice (P<0.0001 and P<0.05), as was DHPG (P<0.01 and P<0.001). Dopamine was similar among groups. Catecholamines were comparable in adrenal glands. No significant differences were found between NET−/− and NET−/+ mice in any specimen; therefore, heterozygotes were excluded from additional experimentation.

Mean Arterial Pressure

Animals were monitored for 3 weeks after implantation of the telemetric device. MAP was elevated in NET−/− mice throughout the entire study, both at rest and with activity (P<0.001 each; Figure 2). A notable elevation of MAP occurred in all animals immediately after surgery. Thereafter, resting MAP declined 2 to 8 days after surgery, ~15 mm Hg (114 to 99 mm Hg) in NET−/− mice (inset, Figure 2) and nearly 25 mm Hg (124 to 99 mm Hg) in NET−/− mice. The adjustment in active pressures was less pronounced, but again, NET−/− mice demonstrated a greater decrease, equal to 14 mm Hg (134 to 120 mm Hg) versus 9 mm Hg in controls (128 to 119 mm Hg).

For the remaining 9 to 21 days, resting MAP stabilized at 99±0.4 and 103±0.6 mm Hg in NET−/− and NET−/+ mice, respectively, and corresponding active pressures were 116±0.3 and 122±0.3 mm Hg. The average overall nightly change to activity increased MAP 17 mm Hg in each genotype throughout the study. Each light/dark phase demonstrated significant genotypic differences over time, which suggests that NET deficiency contributes to elevated MAP.

Heart Rate

The chronotropic effect of NET deficiency was manifested as greater resting HR in NET−/− mice (P<0.05) throughout the study (Figure 3), and genotypic differences at night were highly significant (P<0.0001). Unlike MAP, HR reached maximum values ~5 days after surgery. The resting HR of NET−/− mice began at 560 bpm and rose to 594 bpm, and that in NET−/− mice climbed to 622 bpm from a low of 592 bpm. With nighttime activity, NET−/− mice reached a maximum HR of 709 bpm 5 nights after surgery, an escalation of 50 bpm, and NET−/+ mice increased 36 bpm, analogous to their daytime shift. Overall, the increase in mean HR from the resting to active phase for the entire study was 64±2.6 bpm.
Over the next 7 days, resting HR declined nearly 40 bpm in NET/H11001 mice and 60 bpm in NET/H11002 mice, reaching a virtual plateau of 551±2.8 and 565±5.2 bpm, respectively, after 12 days. During the same period, active HR fell approximately 50 to 55 bpm in both NET/H11001 and NET/H11002 animals, leveling off to corresponding mean values of 607±2.8 and 640±4.5 bpm, with highly significant genotypic differences (P<0.0001). Collectively, these data demonstrate that NET deficiency significantly affects HR, suggesting a prolonged duration of noradrenergic signaling that is particularly evident with sympathetic activation in response to nocturnal locomotion.

**Respiratory Rate**

Diurnal respiratory rates (Figure 4) reveal a postoperative waxing and waning similar to HR. Although incremental
changes over time were less striking, NET−/− mice had consistently lower resting respiratory rates with highly significant genotypic differences (P<0.0001). Active values, however, were nearly equivalent (P=0.1331). Peak respiratory rates at 5 days were 172±5.0 rpm in NET+/+ and 166±11 rpm in NET−/− mice. Corresponding active measurements were 186±4.5 and 193±8.6 rpm.

Resting respiration stabilized at 167±0.9 and 154±1.5 rpm and active values at 181±1.2 and 184±1.7 rpm in NET+/+ and NET−/− mice, respectively. Comparison of the average increase in breathing for each group after transition from rest to activity was highly significant (P<0.0001; inset, Figure 4). The lower resting respiratory rate of NET−/− mice introduces the possibility of diminished central respiratory drive and strongly suggests that NET deficiency differentially affects specific organ systems.

Locomotor Activity
Locomotor activity was evaluated as a possible factor in the elevated hemodynamic measurements of NET−/− mice. Movements at rest did not vary between genotypes (Figure 5), although NET−/− mice were significantly more active for the first 14 nights of the study (P<0.0001). During that time, the activity of NET−/− mice rose from ~7 to 13 cpm, and that of NET+/− mice rose from 10 to 19 cpm. Nocturnal locomotion beyond 2 weeks, however, was indistinguishable. Elevated activity in NET−/− mice during postoperative recovery may be linked to the central effects of transporter deficiency.

Figure 4. Respiratory rates in resting NET−/− mice (○; n=9) were consistently lower than those in NET+/+(●; n=11) mice. When active, NET−/− mice (□) were nearly equivalent to NET+/+ mice (●). The increase in respirations of NET−/− mice (white bar [KO]) was approximately 2-fold greater than that of NET+/+ mice (black bar [WT]) with transition from rest to activity (inset).

Figure 5. Locomotor activity in resting NET−/− (●; n=11) and NET+/+ mice (○; n=9). When active, NET−/− mice (□) showed more movement than NET+/+ mice (●) for the first 2 weeks, but activity was equivalent thereafter.

Discussion
Plasma NE was approximately 2-fold greater in NET−/− than in NET+/+ mice and 1.4 times greater in urine. Higher levels might be expected given that NET is the primary conduit for NE reuptake; however, the central effect of elevated NE is to decrease sympathetic tone, a tenet further supported by measurement of decreased renal sympathetic nerve activity in NET−/− mice (A. Diedrich, MD, PhD, unpublished data, 2003). Reduced neurotransmission, in turn, correlates with decreased NE release.

Plasma dopamine and epinephrine were nearly 3.5 times greater in NET−/− than in NET+/+ mice. Dopamine has a greater affinity for NET than NE itself,1,2,4,2,5 and, in certain brain tissues, NET is the primary means of dopamine clearance.2,3 In nonneuronal tissue such as the adrenal gland, however, NET is immunolocalized intracellulary, and its role in reuptake is considered less significant,26,27 as demonstrated in the present study by equivalent adrenal catechols. Given the modest increase in NE, plasma epinephrine may seem disproportionately high. Despite the likelihood of greater extraneuronal removal of epinephrine by uptake2, which preferentially binds epinephrine,1 only ≤5% of monoamines are normally removed via this route, underscoring the reliance on neuronal reuptake. In addition, NET blockade with desipramine decreases sympathetic nerve activity and NE release while increasing epinephrine release.28,29 Finally, adrenomedullary and sympathetic nerve neurotransmitter release can be triggered by divergent stimuli,28,30 and responsiveness may be skewed by NET deficiency.

Decreased plasma DOPA may represent elevated precursor turnover, increased NE synthesis, and increased tyrosine hydroxylase (TH) activity, as proposed previously by others measuring accumulated L-DOPA in NET−/− mice.19 However, it has been determined that decreased plasma DOPA corresponds to reduced catecholamine synthesis and therefore lower TH activity.31–33 Furthermore, desipramine decreases plasma DOPA levels 20% in rats, demonstrating the negative impact of NET blockade on TH activity.31 Without reuptake of residual NE and dopamine, less substrate is available for intraneuronal oxidation, resulting in lower plasma DHPG. These circumstances are postulated to contribute to decreased NE and DHPG in brain and heart as well. NE in brain homogenates was ~65% that of controls, similar to estimates of 55% to 70% reported in isolated brain tissues,19 whereas DHPG was 40%.

Together, the levels of catecholamines and metabolites in NET−/− mice demonstrate the importance of heterologous uptake by NET in clearance of dopamine in addition to NE. Moreover, these results suggest physiological adaptation to lifelong NET deficiency, including reduced sympathetic outflow and a decreased demand for NE release, reduced vesicular stores, and lower TH activity. In a similar model of
transporter deficiency, TH mRNA, protein, activity, and distribution in NET−/− mice were independently regulated in a tissue-specific manner, which demonstrates the need for further investigation of the mechanisms by which NET controls noradrenergic transmission.34

MAP was elevated immediately after surgery in both genotypes and decreased in a linear fashion thereafter until reaching a plateau at 9 days. The average shift in MAP after transition from rest to activity was equivalent in both genotypes and is in agreement with related studies.35 It might be expected that NET deficiency would result in even greater pressure changes because sympathetic overactivity is recognized as an underlying cause of essential hypertension in humans.36 However, the NET gene has been absent since embryogenesis, and the nearly normal pressures of resting NET−/− mice illustrate developmental adaptation in response to a hyperadrenergic state and the magnitude of sympathetic noradrenergic buffering.

NET-deficient animals exhibit a dramatically elevated HR with wakefulness and activity. Unlike MAP, HRs did not peak until approximately 5 days after surgery. The latent rise in HR may be related to decreased postoperative locomotion and suggests distinct vascular and cardiac adaptations to implantation of the telemetric device, perhaps involving differential engagement of the baroreflex. HR rose 50 bpm in NET-deficient mice, reaching a mean maximum of 709 bpm 5 nights after surgery. Reported HRs in other mice are in the range of 500 to 700 bpm, and at >700 bpm, limited ventricular filling is believed to hamper cardiac output and contractility.37 The present study demonstrates that NET−/− mice have significantly elevated HR compared with NET+/− controls, particularly with nocturnal activity. Furthermore, complete NET loss can propel cardiac performance to levels near or beyond the functional ceiling normally operative in mice and may increase the risk for development of stress-induced or age-related cardiomyopathy.

Respiratory rates followed a postoperative course analogous to HR, reaching maximal levels at 5 days. Surprisingly, the mean respiratory rate was consistently lower in NET−/− mice at rest. With activity, both genotypes were nearly equivalent and, although the trend did not persist, active NET−/− mice respirations were higher for the first 10 to 12 nights of the study. The disparity between respiration and HR in resting animals highlights the different central and peripheral effects of NET deficiency as well as its regional impact. NE normally inhibits activity of the medullary respiratory rhythm generator,37 and episodic hypercapnia induces central NE release and can further inhibit respiratory output.38 Although apnea or arterial CO2 was not measured, central mechanisms may be enhanced in NET-deficient mice, resulting in reduced respirations. Furthermore, noradrenergic regulation is normally limited to bronchodilation and constriction of pulmonary blood vessels.39 Sympathetic input to the heart, on the other hand, is the primary means of stimulating cardiac contractility in mice.35 In the present context, therefore, the central effect of NET deficiency is dominant with regard to respiratory inhibition, whereas in the periphery there is overwhelming stimulation of the heart. With impaired removal of residual NE (and epinephrine), β-adrenergic signaling at the sinoatrial node is apparently prolonged, generating a cumulative effect and driving cardiac contractility upward during activity despite enhanced central modulation.

Nocturnal activity was significantly greater in NET−/− mice for only the first 14 days of the study. Xu et al9 reported lower locomotor activity in NET-deficient mice assessed in the open field, a test paradigm recognized for inducing stress. In the present study, however, activity was monitored in the home cage, an environment designed to be minimally stressful. Activity in the open field tests also became genotypically indistinguishable after habituation to the novel setting.16 This suggests that habituation may also occur, albeit more slowly, after laboratory mice transfer from grouped housing to individual cages and a novel setting.

Collectively, these findings demonstrate that NET deficiency significantly increases extraneuronal levels of NE, dopamine, and epinephrine. The physiological impact of this hyperadrenergic state is perhaps greatest in the periphery and is manifested by elevated blood pressure and excessive tachycardia with wakefulness and physical activity. The diagnostic criteria for orthostatic intolerance includes HR increases >30 bpm with no significant change in blood pressure and increased plasma NE with upright posture.40 Although rodents obviously do not normally maintain a vertical stance, the tachycardia elicited by physical activity in NET−/− mice has striking similarities to the hyperadrenergic cardiovascular phenotype of NET-deficient orthostatic intolerance.12,16,41 Furthermore, the disparity between resting HRs and respiratory rates underscores the tissue-specific impact of NET deficiency. These findings have broad clinical implications for better understanding of the cardiovascular effects of reduced neurotransmitter removal due to pharmacological NET blockade, as well as in disease manifestations such as orthostatic intolerance.

Acknowledgments

We appreciate Jane Wright for providing expert technical assistance and express gratitude to Dr Randy Blakely of the Vanderbilt University Center for Molecular Neuroscience for intellectual contributions.

References

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_Circulation_. 2004;110:1191-1196; originally published online August 30, 2004; doi: 10.1161/01.CIR.0000141804.90845.E6
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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