Norepinephrine transporter function and human cardiovascular disease

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WITH FEW EXCEPTIONS, norepinephrine is the main neurotransmitter released from postganglionic sympathetic neurons in peripheral tissues. Norepinephrine also serves as an important neurotransmitter in the brain. Norepinephrine’s biological effects are mediated through stimulation of pre- and postsynaptic adrenoceptors. In the periphery, norepinephrine increases heart rate, cardiac contractility, vascular tone, renin angiotensin system activity, and renal sodium reabsorption. In contrast, in some brain areas, norepinephrine shuts off centrally generated sympathetic activity. Approximately 80–90% of the released norepinephrine is taken up again through the neuronal norepinephrine transporter (NET). Pharmacological studies with NET inhibitors showed that NET has opposing effects on cardiovascular sympathetic regulation in the brain and in the periphery. Furthermore, NET is involved in the distribution of sympathetic activity between vasculature, heart, and kidney. Genetic NET dysfunction is a rare cause of the postural tachycardia syndrome. The condition is characterized by excessive adrenergic stimulation of the heart, particularly with standing. Conversely, NET inhibition may be beneficial in hypoadrenergic states, such as central autonomic failure or neurally mediated syncope, which results from acute sympathetic withdrawal. Biochemical studies suggested reduced NET function in some patients with essential hypertension. Furthermore, cardiac NET function appears to be reduced in common heart diseases, such as congestive heart failure, ischemic heart disease, and stress-induced cardiomyopathy. Whether NET dysfunction is a consequence or cause of progressive heart disease in human subjects requires further study. However, studies with the nonselective NET inhibitor sibutramine suggest that reduced NET function could have an adverse effect on the cardiovascular system. Given the widespread use of medications inhibiting NET, the issue deserves more attention.

norepinephrine; norepinephrine transporter; cardiovascular; autonomic nervous system; blood pressure regulation

Schroeder C, Jordan J. Norepinephrine transporter function and human cardiovascular disease. Am J Physiol Heart Circ Physiol 303: H1273–H1282, 2012.—Approximately 80–90% of the norepinephrine released in the brain or in peripheral tissues is taken up again through the neuronal norepinephrine transporter (NET). Pharmacological studies with NET inhibitors showed that NET has opposing effects on cardiovascular sympathetic regulation in the brain and in the periphery. Furthermore, NET is involved in the distribution of sympathetic activity between vasculature, heart, and kidney. Genetic NET dysfunction is a rare cause of the postural tachycardia syndrome. The condition is characterized by excessive adrenergic stimulation of the heart, particularly with standing. Conversely, NET inhibition may be beneficial in hypoadrenergic states, such as central autonomic failure or neurally mediated syncope, which results from acute sympathetic withdrawal. Biochemical studies suggested reduced NET function in some patients with essential hypertension. Furthermore, cardiac NET function appears to be reduced in common heart diseases, such as congestive heart failure, ischemic heart disease, and stress-induced cardiomyopathy. Whether NET dysfunction is a consequence or cause of progressive heart disease in human subjects requires further study. However, studies with the nonselective NET inhibitor sibutramine suggest that reduced NET function could have an adverse effect on the cardiovascular system. Given the widespread use of medications inhibiting NET, the issue deserves more attention.

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Genetic Influences on NET Function and Familial NET Deficiency

POTS is a relatively common condition primarily affecting women in their reproductive years (57, 62, 63). POTS is defined as an exaggerated increase in heart rate of >30 beats/
min upon standing without orthostatic hypotension, together with orthostatic symptoms persisting for more than 3 mo (38). Elevated plasma norepinephrine concentrations are common in these patients (57, 62). In a POTS patient and her monozygotic twin sister, extensive physiological, biochemical, and pharmacological testing suggested reduced neuronal norepinephrine uptake. The systemic clearance of radioactively labeled norepinephrine was substantially reduced. Furthermore, the patient showed attenuated norepinephrine release during tyramine infusion. Tyramine requires a functioning NET to enter adrenergic neurons and to release norepinephrine. Together, these findings suggested that hyperadrenergic symptoms in the twin pair may have been secondary to impaired norepinephrine uptake.

The human NET gene (SLC6A2, NET-1) was isolated and cloned in 1991 (97). The gene is located on chromosome 16q12.2 and encodes a 617 amino acids protein (16). The gene is located on chromosome 16q12.2 and encodes a 617 amino acids protein (16). The g237c mutation is associated with 145 healthy volunteers (67). These NET variants were significantly associated with the pressor response during exercise, but not with heart rate or plasma catecholamine changes. Among the six polymorphisms described (66, 124), splice variants for the NET gene product have also been described (316). Hahn et al. (45) studied protein expression, trafficking, and norepinephrine reuptake function in a number of NET polymorphisms leading to an amino acid exchange. The investigators also tested whether or not these polymorphisms responded normally to pharmacological inhibition with the prototypical tricyclic antidepressant desipramine. Six out of ten polymorphisms altered NET function. The A369P polymorphism was retained intracellularly and lacked norepinephrine transport activity much like the A457P mutation in patients with NET deficiency. A369P and N292T polymorphisms exerted a dominant negative effect on wild-type NET. In contrast, the F528C polymorphism increased norepinephrine uptake by 30%. Remarkably, norepinephrine uptake through the F528C NET variant was not blocked by desipramine and was insensitive to protein kinase C-mediated downregulation. Allele frequencies as high as 5% for A369P and 10% for N292T and F528C have been identified in the literature (45). However, these numbers are controversial. Recently, the frequency of two other NET gene variants (T182C and A3081T) that alter NET promoter activity has been reported as 44% and 59%, respectively, in a sample of 145 healthy volunteers (67). These NET variants were significantly associated with the pressor response during exercise, but not with heart rate or plasma catecholamine changes. Splice variants for the NET gene product have also been described (66, 124).
Finally, the NET gene may be subject to epigenetic modification (7, 48). Recently, Bayles et al. (8) reported reduced NET expression in leukocytes from POTS patients that could not be explained by genetic variation of the NET gene. NET promoter methylation did not differ between groups. However, NET gene chromatinization differed markedly between POTS patients and healthy subjects, indicating extensive histone modification that might explain differences in NET expression (8).

Given the known efficacy of medications inhibiting NET in the treatment of depression, several groups tested the hypothesis that common NET polymorphisms are associated with psychiatric disorders. These studies yielded controversial results with some indicating an association with depression (44, 109), panic disorder (19, 73), attention deficit disorder (12, 47), and social phobia (40), whereas others did not (96, 105). However, increased sympathetic tone has been identified as a likely contributor to the increased cardiovascular risk in various psychiatric diseases (33), particularly depression (41). Biochemical data suggest that reduced cardiac NET function might be present at least in some patients with major depression (6). It is tempting to speculate that disordered NET function in depression is due to altered physiological regulation of epigenetic mechanisms rather than genetics. If so, these mechanisms could provide yet another target for treating depression as well as the associated disordered sympathetic regulation.

Studies on NET genetics and cardiovascular disease are scarce. However, an association between a polymorphism in the promoter 3 region of the NET gene and hypertension has been found in Japanese and Caucasian populations (95, 136). In another study, a different NET polymorphism was associated with hypertension in patients with type 2 diabetes (70). Since many association studies have not been replicated, these observations should be interpreted cautiously. However, biochemical and pharmacological studies suggesting reduction in neuronal norepinephrine uptake in some patients with essential arterial hypertension are reassuring (31, 111).

**Pharmacological NET Inhibition: Insight in Human Physiology**

Genetic studies, particularly studies in patients with NET deficiency, strongly suggested that NET may contribute to human cardiovascular disease. However, much of the information on how NET affects the human cardiovascular system has been obtained in pharmacological studies with selective and nonselective NET inhibitors. Together, these studies suggest that NET regulates the cardiovascular system through actions in both peripheral tissues and the brain.

In healthy subjects, short-term pharmacological NET inhibition increases resting blood pressure and heart rate in the supine position (114). Paradoxically, the pressor response to sympathetic stimuli, such as the cold pressor test, is decreased with NET inhibition (114). The combined serotonin and norepinephrine uptake inhibitor sibutramine, an adjunctive obesity treatment, elicits similar acute hemodynamic responses, both in healthy subjects (10) and in obese patients (11, 52). Thus NET inhibition can inhibit as well as stimulate sympathetic responses.

When NET inhibitors are infused intra-arterially into the forearm in doses insufficient to cause a systemic response, norepinephrine in forearm veins is increased (21). This peripheral mechanism tends to increase sympathetic responses. In contrast, systemic NET inhibition reduces venous norepinephrine concentrations in the supine position (113) as well as systemic norepinephrine spillover (34). NET inhibition profoundly decreases resting sympathetic nerve activity (Fig. 2) (34, 128). Finally, NET inhibition shifts the sympathetic baroreflex curve toward a higher blood pressure without affecting its slope (128). These changes in norepinephrine turnover and sympathetic nerve traffic are consistent with central nervous sympathetic inhibition. Animal studies suggest that sympathetic inhibition elicited by NET blockade may be mediated through $\alpha_2$-adrenoceptor stimulation in the brain (30).

Taken together, the effect of NET inhibition on blood pressure results from a sympatholytic actions in the brain, which tends to lower pressure, and a stimulatory effect in the periphery, which tends to raise pressure. The idea is supported by the observation that patients with increased centrally generated sympathetic activity experience a lesser increase in blood pressure than patients with lower sympathetic activity when treated with a NET inhibitor (51). In patients with increased sympathetic activity, the sympatholytic effect opposes peripheral actions of the NET inhibitor. In contrast, in patients with low sympathetic activity, sympathetic activity cannot be decreased further, such that the peripheral NET inhibitor action prevails. In accordance with this concept, selective NET blockade with atomoxetine induced a substantial increase in blood pressure in patients with central autonomic failure (119). In this condition, peripheral adrenergic neurons are disconnected from brain stem input (117) such that the peripheral stimulatory effect of NET inhibition is unmasked.

NET inhibition changes norepinephrine turnover in an organ-specific fashion. Studies with the nonselective NET inhibitor desipramine showed reductions in forearm and renal norepinephrine spillover (34). In contrast, cardiac norepinephrine spillover increased with desipramine (34). These changes in the distribution of adrenergic activity between organs lead to corresponding changes in organ function. For example, NET inhibition attenuates supine and upright plasma renin activity and angiotensin II concentrations (80). NET inhibition also attenuates renal vasoconstriction with standing (80). In contrast, NET inhibition augments the increase in plasma norepinephrine during orthostatic stress (113) and profoundly increases upright heart rate, thus mimicking the hemodynamic abnormalities in POTS patients (Fig. 3) (114). NET inhibition also raises heart rate with exposure to gravitational stress in a human centrifuge (126). Yet, NET inhibition prevents neurally mediated presyncope and syncope during head-up tilt testing (Fig. 4), which are characterized by acute sympathetic withdrawal (113).

We observed considerable sex differences in the hemodynamic response to short-term NET inhibition. The increase in resting blood pressure, which was mainly due to an increase in cardiac output, was threefold larger in men than in women. Furthermore, NET inhibition augmented upright heart rate to a greater extent in men than in women, which could indicate reduced cardiac NET activity in women (112). A subsequent study indicated that fluctuations in female sex hormones during...
NET Dysfunction and Chronic Heart Disease

Recent studies suggest an association between chronically altered NET function and heart disease. Heart diseases may alter norepinephrine uptake. Indeed, norepinephrine uptake from the synaptic cleft through NET is an active, energy-dependent process (14), which could be altered in conditions characterized by limited oxygen and substrate supply. It is also true that the menstrual cycle is associated with changes in the cardiovascular response to NET inhibition (85).

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Fig. 2. Reboxetine plasma concentrations (top, ■), systolic and diastolic blood pressure (middle, ▲), heart rate (middle, ○), and muscle sympathetic nerve activity (bottom, ▼) in the supine position in 6 healthy male controls after a single oral dose of 8 mg reboxetine (▲P < 0.05, ★★P < 0.01, repeated-measures ANOVA, Dunnett’s posttest). AU, arbitrary units. Selective NET inhibition by reboxetine increases systolic blood pressure and heart rate after 90 min. Muscle sympathetic nerve activity is profoundly decreased 60 min after reboxetine ingestion. Figure redrawn from previously published work with permission (128).

Fig. 3. Heart rate [in beats/min (bpm)] in the supine position (0°) and with increasing orthostatic stress during incremental head-up tilt in 18 healthy controls with placebo (○) and selective pharmacological NET inhibition with reboxetine (▲). NET inhibition increased heart rate in the supine position (#P < 0.001, paired t-test). More strikingly, the increase in heart rate during incremental orthostatic stress was profoundly augmented with NET inhibition (★★★P < 0.001, ANOVA). Figure redrawn from previously published work with permission (114).

Fig. 4. Individual differences in tilt tolerance measured as the tolerated time during head-up tilt testing between pharmacological NET inhibition and placebo in 51 healthy controls. In subjects who tolerated the full tilt test protocol both on placebo and with NET inhibition, the difference is 0. The solid vertical lines indicate the mean values and the boundaries of the 95% confidence interval. Figure redrawn from previously published work (113) with permission.

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possible that altered norepinephrine uptake promotes heart disease. Since many clinical investigations in this field rely on correlations, cause and effect are difficult to distinguish from each other.

The idea that excessive synaptic norepinephrine concentrations may promote heart disease through NET dysfunction is not new. More than two decades ago, profoundly diminished norepinephrine uptake was described in hypertrophic cardiomyopathy patients (15). Excessive catecholamine concentrations can acutely damage the heart (79) and contribute to myocardial electrical instability, thereby predisposing to cardiac dysrhythmias (122). Indeed, excessive catecholamine release in patients with pheochromocytoma can induce a cardiac myopathy (42). Evidence for excessive adrenergic activity in the heart has been reported in myocardial infarction (1), unstable ischemic heart disease (81), stress-induced cardiomyopathy (71), and congestive heart failure (104). In patients with myocardial infarction, norepinephrine spillover was increased in those who subsequently developed heart failure (1). Excessive plasma norepinephrine concentrations predict a poor prognosis in patients with severe congestive heart failure (98). Yet, the most persuasive finding linking excessive cardiac sympathetic activity with heart disease progression is the beneficial effect of β-adrenoceptor blockers in congestive heart failure (36, 58) and after acute myocardial infarction (39). β-Adrenergic blockade does not seem to decrease cardiac sympathetic tone itself (5), but rather block its deleterious effects on the myocardium.

Excessive sympathetic neural drive to the heart appears to be the dominant mechanism leading to adrenergic overactivation in heart failure. However, NET dysfunction could further exacerbate sympathetically mediated heart disease. In addition, NET dysfunction could limit the physiological adjustment in cardiac sympathetic through depletion of norepinephrine stores. Cardiac tracers applied in clinical imaging such as [123I]-meta-iodobenzylguanidine ([123I]-MIBG), [18F]-fluorodopamine, [11C]-hydroxyephedrine, and [3H]-norepinephrine, are all dependent on iodobenzylguanidine ([123I]-MIBG), [18F]-fluorodopamine, [11C]-hydroxyephedrine, and [3H]-norepinephrine, are all dependent on uptake into cardiac adrenergic nerve terminals through NET. Decreased tracer uptake has been observed in patients with myocardial infarction (68, 120, 121), stress-induced cardiomyopathy (17, 23, 56, 88, 101, 110, 127, 135), and congestive heart failure (2, 84). In a small study in patients with arrhythmogenic right ventricle cardiomyopathy cardiac uptake of [11C]-hydroxyephedrine tended to be attenuated (133). Finally, in idiopathic ventricular arrhythmia patients, the NET gene expression was downregulated in the septal wall of the right ventricular outflow tract (49). Decreased cardiac radiotracer uptake is associated with a poor prognosis in patients with ischemic heart disease as well as in patients with dilated or hypertrophic cardiomyopathy (90).

Stress-induced cardiomyopathy has been reported after overdosing with the unspecific NET inhibitor nortriptyline (27), the combined selective NET and serotonin transporter (SERT) inhibitors venlafaxine (22, 91, 130), and milnacipran (74), as well as with amphetamines that also exert NET inhibition (3). Among other pharmacological actions, cocaine inhibits NET function (37). Cocaine abuse can induce cardiomyopathies (4).

Excessive norepinephrine release in congestive heart failure is not adequately matched by norepinephrine reuptake because of either downregulation of NET (13, 65, 75) or decreased NET efficiency (29). The previously mentioned reduction in [123I]-MIBG uptake in these patients also indicates an impaired norepinephrine reuptake and storage system (2, 84). Impaired [123I]-MIBG uptake occurs early in the course of congestive heart failure regardless of underlying etiologies and affects both prognosis (55) and treatment (64). An eightfold increase in cardiac norepinephrine spillover was associated with moderately reduced norepinephrine clearance in congestive heart failure patients (83). In human cardiac tissue obtained during cardiac transplantation, NET expression and norepinephrine reuptake were reduced (9, 13). Finally, preventing NET down regulation by adenosiviral transfer of NET gene in an animal model of heart failure increased cardiac norepinephrine content and improved heart function (89). Interestingly, the treatment response in dilated cardiomyopathy is dependent on NET polymorphisms, with patients exhibiting the T182C genotype being resistant to β-adrenoceptor blocker therapy (93).

Moreover, together with two distinct adrenoceptor genotypes the T182C genotype may negatively affect the outcome in dilated cardiomyopathy patients (94). Given the high prevalence of the T182C genotype (67), this finding might be of importance for a large number of heart failure patients. Recent findings suggest that treatment for heart failure by both, combined β- and α1-adrenergic blockade with carvedilol (78) or mechanical unloading by means of a left ventricular assist device (28) may improve norepinephrine reuptake in patients with heart failure. Together, these findings suggest that NET dysfunction may, indeed, play a major role in cardiac disease. In fact, heart disease progression and recuperation may be affected.

Long-Term Cardiovascular Response to Pharmacological NET Inhibition

Numerous drugs inhibit NET function. Older substances, such as tricyclic antidepressants, rather unselectively inhibit a range of monoamine transporters and metabolizing enzymes. Newer agents are more selective for NET and/or other monoamine transporter, such as the SERT. Most NET and combined NET/SERT inhibitors are approved as antidepressants and are among the most frequently prescribed drugs. Recently, more than 10% of U.S. Americans aged 12 or older reported antidepressant use with a clear preponderance in females (102). Some NET inhibitors are approved for other indications: atomoxetine is licensed for use in patients with attention deficit syndrome, whereas duloxetine is prescribed in painful diabetic neuropathies, a condition associated with increased cardiovascular risk, and in fibromyalgia (50). Finally, the NET/SERT inhibitor sibutramine has been utilized in the treatment of obesity.

Theoretically, NET inhibition could damage the cardiovascular system directly through actions within the target tissues and indirectly by increasing blood pressure. Recent data suggest that the use of tricyclic antidepressants as well as NET inhibitors is associated with increases in blood pressure (76). Data on long-term influences of pharmacological NET inhibition on blood pressure have been obtained with a combined NET/SERT inhibitor, the weight loss drug sibutramine. For example, combined analysis of two placebo-controlled trials showed no significant change in systolic blood pressure over a 48-wk period (61). Diastolic blood pressure was 1.1 mmHg
increased with sibutramine. The blood pressure response was not exacerbated in patients with grade 1 or 2 hypertension or in patients with isolated systolic hypertension, and the pressure response to sibutramine treatment varied substantially between patients. Nonetheless, there is evidence for adverse influences of sibutramine on cardiovascular morbidity. In the recent Sibutramine Cardiovascular Outcome Trial (SCOUT) (59), overweight or obese patients with type 2 diabetes mellitus and/or a medical history of cardiovascular disease were randomized to treatment with sibutramine or placebo. Based on study results of an increased risk for nonfatal heart attack and stroke with sibutramine, the European Medicines Agency recommended suspending its license in Europe (35).

The interpretation of cardiovascular morbidity data in NET inhibitor-treated patients is complicated by the fact that the disease leading to NET inhibitor prescription may be associated with altered cardiovascular disease risk. For example, depression is an independent risk factor for cardiovascular disease (53). Conversely, patients with heart disease are prone to experience depression (20). Most studies investigating whether antidepressants affect cardiovascular risk involved tricyclic antidepressants, which interact with many monoamine transporters and receptors, and selective serotonin reuptake inhibitors. Some (24, 87, 118, 129, 134) but not all (82, 86) showed an increased risk particularly in patients on tricyclic antidepressants. Serotonin reuptake inhibitors, such as sertraline, are considered safe, even in patients with preexisting cardiovascular disease (60, 99).

With the consideration of the widespread use of NET inhibitors including in populations at high cardiovascular risk, data on long-term cardiovascular safety are surprisingly scarce. The issue of possible negative effects of NET inhibitors on cardiovascular morbidity and mortality is not conclusively solved. A number of epidemiological studies showed an association between antidepressant use and cardiovascular risk (69, 123, 131, 132). Discrepancies in populations and methodological approaches may explain why other studies did not find such association (25, 43). Recently, a disproportionality analysis failed to show an association between the antidepressants inhibiting NET and cardiomyopathy risk (103). However, findings from cases of drug overdosing suggest that both NET inhibition with or without additional SERT inhibition can be cardiotoxic with tachycardia and increases of blood pressure being the most frequent findings (54, 77). In case reports, overdosing and, rarely, normal doses (107, 115) of NET/SERT inhibitors have been linked with frank cardiomyopathy (22, 27, 74, 91, 130).

NET inhibitors may also interfere with the reuptake of other biogenic amines, such as dopamine, which could affect toxicity. Furthermore, older unspecific selections like tricyclic antidepressants are well known for their cardiac side effects likely through inhibition of cardiac ion channels rather than NET inhibition. However, newer NET inhibitors are much more selective and less likely to affect other ion channels or other monoamine transporters. Norepinephrine’s cardiotoxic potential compared with other biogenic amines (18) suggests that excessive local norepinephrine concentrations could play an important role in NET inhibitor mediated cardiotoxicity.

Conclusions

NET has a pivotal role in the regulation of synaptic norepinephrine in the brain and in peripheral tissues. Pharmacological studies with NET inhibitors showed that NET has opposing effects on cardiovascular sympathetic regulation in the brain and in the periphery. Furthermore, NET is involved in the distribution of sympathetic activity between vasculature, heart, and kidney. There is evidence that genetic NET dysfunction is pathophysiologically involved at least in some cases of “hyperadrenergic” POTS. Conversely, NET inhibition may be beneficial in “hypoadrenergic” states such as central autonomic failure or neurally mediated syncope. Evidence for impaired cardiac NET function has been obtained in a range of cardiac conditions, both common and chronic, e.g., congestive heart failure, and less frequent but acute, such as stress-induced, cardiomyopathy. Whether NET dysfunction is the cause or merely a consequence of heart disease in humans requires further study. However, there is compelling evidence that reduced NET function could have an adverse effect on the cardiovascular system, either through direct norepinephrine-mediated cardiotoxicity or indirectly through changes in blood pressure. Given the widespread use of medications inhibiting NET, the issue deserves more attention.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

C.S. and J.J. conception and design of research; C.S. performed experiments; C.S. and J.J. analyzed data; C.S. and J.J. interpreted results of experiments; C.S. prepared figures; C.S. drafted manuscript; C.S. and J.J. approved final version of manuscript; J.J. edited and revised manuscript.

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