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The neuronal noradrenaline transporter, anxiety and cardiovascular disease

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Abstract

Panic disorder can serve as a clinical model for testing whether mental stress can cause heart disease. Potential neural mechanisms of cardiac risk are the sympathetic activation during panic attacks, continuing release of adrenaline as a co-transmitter in the cardiac sympathetic nerves, and impairment of noradrenaline neuronal reuptake, augmenting sympathetic neural responses.

The phenotype of impaired neuronal reuptake of noradrenaline: an epigenetic mechanism? We suspect that this phenotype, in sensitizing people to heart symptom development, is a cause of panic disorder, and by magnifying the sympathetic neural signal in the heart, underlies increased cardiac risk. No loss of function mutations of the coding region of the norepinephrine transporter (NET) are evident, but we do detect hypermethylation of CpG islands in the NET gene promoter region. Chromatin immunoprecipitation methodology demonstrates binding of the inhibitory transcription factor, MeCP2, to promoter region DNA in panic disorder patients.

Cardiovascular illnesses co-morbid with panic disorder. Panic disorder commonly coexists with essential hypertension and the postural tachycardia syndrome. In both of these cardiovascular disorders the impaired neuronal noradrenaline reuptake phenotype is also present and, as with panic disorder, is associated with NET gene promoter region DNA hypermethylation. An epigenetic 'co-morbidity' perhaps underlies the clinical concordance.

Brain neurotransmitters. Using internal jugular venous sampling, in the absence of a panic attack we find normal norepinephrine turnover, but based on measurements of the overflow of the serotonin metabolite, 5HIAA, a marked increase (six to sevenfold) in brain serotonin turnover in patients with panic disorder. This appears to represent the underlying neurotransmitter substrate for the disorder. Whether this brain serotonergic activation is a prime mover, or consequential on other primary causes of panic disorder, including cardiac sensitization by faulty neuronal noradrenaline reuptake leading to cardiac symptoms and the enhanced vigilance which accompanies them, is unclear at present.

Keywords

NET gene, epigenetics, DNA methylation, hypertension, panic disorder, postural tachycardia syndrome, noradrenaline, serotonin

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Introduction

Some people are subject to episodes of recurring, often inexplicable anxiety. These attacks typically are very unpleasant, and accompanied by physical symptoms such as sweating, palpitations, tremor and a sensation of suffocation. Recurring attacks over a period of months, or in many cases years, form the basis for the diagnosis of panic disorder (American Psychiatric Association, 1994). This is a distressing condition, and can lead to social avoidance behaviour, in some instances so extreme that the sufferer will never leave the home.

It has until recently been felt that although panic disorder was distressing and disabling, it did not constitute a risk to life. Sufferers often fear that they have heart disease, because of the nature of their symptoms, but in the past have been reassured that this is not the case. Recent epidemiological studies, however, indicate that there is an increased risk of myocardial infarction and sudden death in patients with panic disorder (Kawachi *et al.*, 1994b; Kawachi *et al.*, 1994a). The cause is not known, but possibly involves activation of the sympathetic nerves of the heart, predisposing to disturbances of cardiac rhythm and possibly coronary artery spasm.

Our own extensive clinical experience with the cardiological management of panic disorder sufferers has provided case material which indicates the range of cardiac complications which occur. During panic attacks we have documented, variously, triggered cardiac arrhythmias, recurrent emergency room attendances with angina and electrocardiogram (ECG) changes of ischaemia, coronary artery spasm documented in panic attacks occurring during coronary angiography and myocardial infarction associated with coronary spasm and thrombosis (Mansour *et al.*, 1998).

The sympathetic nervous system and adrenal medulla under resting conditions in panic disorder

Sympathetic nervous activity and epinephrine secretion rates

Adrenal medullary secretion of epinephrine, measured by isotope dilution, is typically normal (Wilkinson *et al.*, 1998). Multi-unit sympathetic nerve firing rates measured directly by microneurography, in the sympathetic outflow to the skeletal muscle vasculature, are also normal in untreated, resting patients with panic disorder (Wilkinson *et al.*, 1998). We do detect a unique pattern of abnormal single fibre sympathetic nerve firing in panic disorder (Lambert *et al.*, 2006), which takes the form of increased probability of multiple nerve firings per heart beat. Salvos of nerve firings like this enhance effector organ responses.

Epinephrine co-transmission in sympathetic nerves

Release of epinephrine from the heart, possibly as an accessory sympathetic neurotransmitter, has been demonstrated in patients with panic disorder (Wilkinson *et al.*, 1998; Esler *et al.*, 2004). It is probable that during the surges of epinephrine secretion accompanying panic attacks, this process loads the sympathetic neuronal vesicles with epinephrine, which is continuously co-released with norepinephrine in the interim periods between attacks. A possible alternative mechanism underlying sympathetic neural epinephrine release is the occurrence of *in situ* synthesis of epinephrine, through activation within the heart of the epinephrine synthesizing enzyme phenylethanolamine-n-methyltransferase (PNMT), perhaps induced by repeated cortisol responses during panic attacks. In experimental models of stress, PNMT activation in the heart and other extra-adrenal organs has been demonstrated (Kvetnansky *et al.*, 2004).

Reduction in neuronal norepinephrine reuptake by sympathetic nerves

Each pulse of the sympathetic neural signal is terminated in tissues primarily by reuptake of the released norepinephrine into the sympathetic varicosity via the norepinephrine transporter (Eisenhofer *et al.*, 1996). The most precise analysis of human sympathetic nerve reuptake of noradrenaline can be achieved with analysis of tracer noradrenaline kinetics (Rumantir *et al.*, 2000). Measurement of the extraction of infused tritiated noradrenaline in passage through the heart, and its intraneuronal conversion to tritiated 3,4-dihydroxyphenylglycol (DHPG) allows direct quantification of neuronal noradrenaline reuptake. Application of this methodology in panic disorder patients has enabled us recently to demonstrate in them the phenotype of impaired neuronal reuptake of noradrenaline by sympathetic nerves (Esler *et al.*, 2004) (Fig. 1). Such an abnormality would be expected to magnify sympathetically mediated responses, particularly in the heart where norepinephrine inactivation is so dependent on neuronal reuptake (Eisenhofer *et al.*, 1996). A defect in noradrenaline reuptake might be expected to have a genetic origin, but to this point we have not identified any loss of function coding region mutations in the noradrenaline transporter (*NET*) gene. Epigenetic changes in the *NET* gene possibly underlying this phenotype of impairment of neuronal noradrenaline reuptake in panic disorder are discussed below.

Sympathetic nervous and adrenal medullary changes during a panic attack

Heart rate and blood pressure increase during a panic attack, primarily due to sympathetic nervous system activation and adrenal medullary secretion of epinephrine (Wilkinson *et al.,* 1998).

Sympathetic nerve firing and secretion of epinephrine

When recorded directly by microneurography, the size of multi-unit sympathetic bursts increases remarkably during a panic attack, without any increase in firing rate (Wilkinson *et al.*, 1998) (Fig. 2), presumably by recruitment of additional firing fibres. This response is qualitatively different from that seen during laboratory mental stress with stimuli such as difficult mental arithmetic,

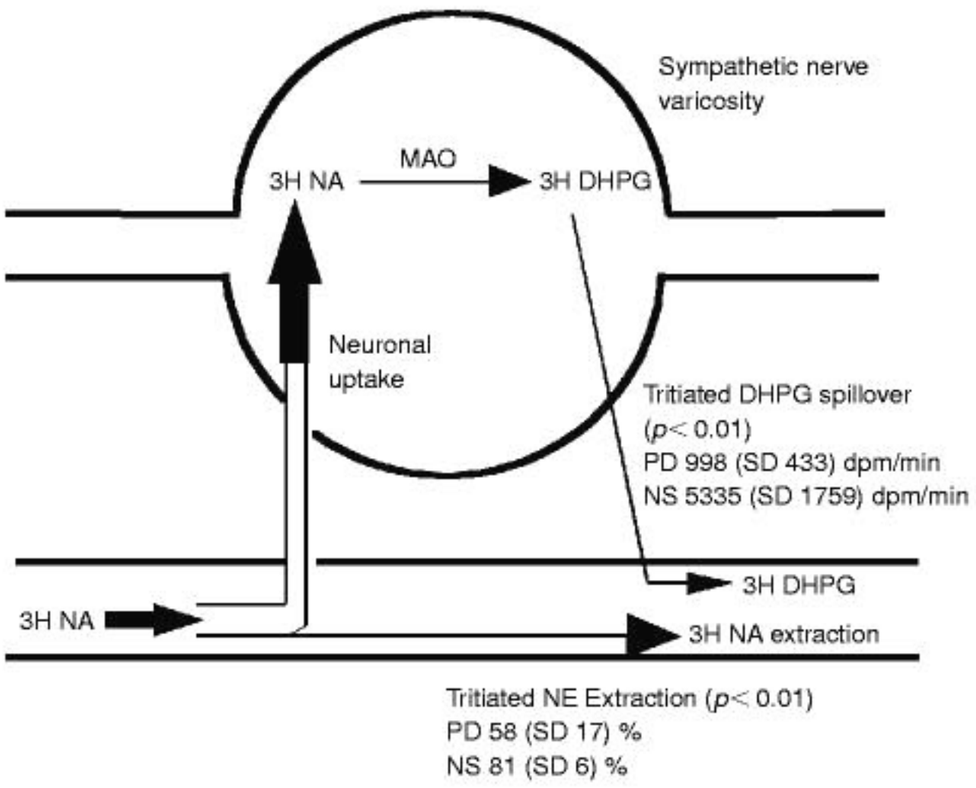

Figure 1 Representation of transcardiac processing of tritiated norepinephrine (3H NE), during an intravenous infusion of the radiotracer. The majority of tritiated norepinephrine was removed from plasma via neuronal uptake by sympathetic nerves. Within sympathetic nerves, 3H NE is metabolised to tritiated 3,4-dihydroxyphenylglycol (3H DHPG) by monoamine oxidase (MAO), with some subsequent release into the venous circulation. Tritiated norepinephrine uptake by the heart was reduced in 21 panic disorder patients compared with healthy subjects ($p < 0.01$). In parallel, release of tritiated DHPG into the coronary sinus venous drainage of the heart was lower, ($p < 0.01$). These findings document the presence of the impaired neuronal norepinephrine reuptake phenotype in panic disorder

where muscle sympathetic nerve activity increases little if at all. During a panic attack, the secretion of epinephrine increases two- to sixfold (Wilkinson *et al.*, 1998).

Release of neuropeptide Y

With the pronounced activation of the cardiac sympathetic outflow occurring during a panic attack, neuropeptide Y (NPY) is co-released from the cardiac sympathetic nerves and appears in measurable quantities in coronary sinus venous blood (Esler *et al.*, 2004).

Neural mechanisms mediating cardiac risk during a panic attack

Release of epinephrine as a co-transmitter from cardiac sympathetic nerves, and activation of the sympathetic nervous system during panic attacks, augmented by the flaw in neuronal reuptake of noradrenaline, are probable mediating mechanisms. Further, a unique pattern of single fibre sympathetic nerve firing in panic

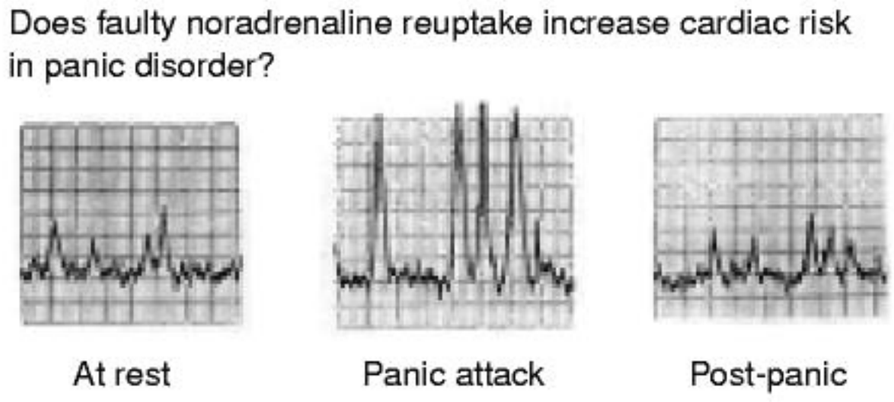

Figure 2 The amplitude of multi-unit sympathetic nerve discharges, shown here in the sympathetic traffic to skeletal muscle blood vessels, increases during a panic attack. An increase in sympathetic outflow of this magnitude to the heart also, coupled with impairment of neuronal reuptake of the transmitter, could produce a potentially arrhythmogenic level of cardiac sympathetic stimulation

disorder (Lambert *et al.*, 2006), taking the form of salvos of nerve firings, enhances effector organ responses (Kunimoto *et al.*, 1992) and, again, would predispose to the development of cardiac arrhythmias. Neuropeptide Y is released from the sympathetic nerves of the heart into the coronary sinus during the sympathetic activation accompanying panic attacks (Esler *et al.*, 2004). NPY has the capacity to cause coronary artery spasm (Hass, 1998).

An epigenetic basis for the impaired neuronal noradrenaline reuptake phenotype in panic disorder?

Analysis of the NET gene

We have not detected loss of function mutations in the *NET* gene coding regions in panic disorder patients. The *NET* gene promoter region is rich in CpG dinucleotides, potentially rendering it susceptible to methylation-related gene silencing. We investigated this possibilty, searching also for the presence of an inhibitory transcription factor, MeCP2, which causes gene silencing by binding to heavily methylated promoter region DNA (El-Osta and Wolffe, 2000; Harikrishnan *et al.*, 2005). Research participants gave their informed consent to the research procedures, which were approved by the Alfred Hospital Research Ethics Committee.

Detection of mutations within the NET gene

Genomic DNA was extracted from leukocytes and ethanol precipitated using a commercial kit following the manufacturer's recommendations (Promega, Madison, WI, USA). The promoter region and exon 9 of the *NET* gene were amplified with the use of the polymerase chain reaction and sense and antisense primers. Two

sense primers for the promoter region were used, 5' CTACCCT-GCCCATAAATAACAGAG 3' and 5' GAGTCCCCCAGATCC-CTGGGAACC 3' with a shared antisense primer, 5' TCCCAGGCAGACCTAGCCCTGTCCC 3'. The sense and antisense primers for exon 9 were, 5' GAAGGCAGGACGTGCT-GATT 3' and 5' GATGCTGGATCCTGCATTGT 3', respectively. The amplified products were purified with the MinElute PCR and Gel Extraction Purification Kit (Qiagen, Hilden, Germany) and sequenced with BigDye terminator chemistry (Applied Biosystems, California, USA) on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, California, USA).

Methylation analysis

Genomic NA was subjected to sodium bisulfite modification protocols using CpGenome DNA Modification kit following the manufacturers recommendations (Chemicon International, Temecula, CA, USA). Methylation (MSP) and unmethylated (USP) primers to the *NET* promoter were designed using Methprimer software for bisulfite-conversion-based methylation specific PCR (MSP) (Li and Dahiya, 2002). Criteria for MSP primer selection were used to identify regions of DNA longer than 100 nucleotides that had G + C content greater than 50% and an observed/expected ratio of CpG dinucleotides greater than 0.6. The quantitation of methylation status was performed using ABI Prism 7700 Sequence Detection System. PCR amplification was performed in 96-well optical plates with a 20μl reaction volume consisting of 50 pmol of forward and reverse primers and 1X SYBR^R Green PCR Master Mix (applied Biosystems, California, USA). Reactions were incubated for 2 min at 50°C, then 10 min at 95°C followed by 40–50 cycles of 95°C/15 sec and 60°C/60 sec. The USP primers were 5'-AATTGTTTAATAGTTTGTTGGTTG T-3' (forward) and 5'-CCAAAACTAAAATCTACATCACATC-3' (reverse) and the MSP primers 5' –AATTGTT-TAATAGTTCGTTGGTCGT-3' (forward) and 5'-AAAAC-TAAAATCTACGTC GCGTC-3' (reverse). The relative level of methylated DNA in each sample was calculated as per cent methylation using the following formula: (Ct*m*/(Ct*m* + Ct*u* X 100)) where Ct refers to the cycle number at which the fluorescence signal crosses a detection threshold and Ct*m* is the methylated and Ct*u* is the unmethylated values. We applied a cut-off value of 9%, which was the average percentage of methylation in the peripheral blood lymphocytes samples.

Chromatin immunopurification for MeCP2 on NET gene

DNA sonication and chromatin immunoprecipitations were performed for enrichment of MeCP2- co-repressor complex (MeCP2 antibody courtesy P.L. Jones, Illinois, USA). Briefly, soluble chromatin fractions were resuspended in dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl [pH 8.1], 167 mM NaCl) for immunoprecipitation of MeCP2 (El-Osta *et al.,* 2002). Binding was performed with protein A/G agarose beads, which were washed and the immuno-complexes separated from DNA by elution with 1% SDS, 0.1 M $NaHCO_3$. Resuspended DNA was amplified by PCR using the following primer sequences, *NET* ChIP 5' GCCGCCTATTTGCAGCACT 3' (forward) and *NET* ChIP 5' GCGCGCACTCCCTTTCTAT 3' (reverse) and HotStarTaq amplification kit (1.5mM MgCl2, 300μM 4dNTP's and 0.5 U Taq DNA polymerase) (Invitrogen, Victoria, Australia). Cycling parameters consisted of 95°C/60sec followed by 32 cycles of 95°C/60sec, 58°C/60sec and 72°C/60sec and an extension at 72°C/10min performed on a GeneAmp 9700 PCR System (ABI, CA, USA). Samples were size fractionated by polyacrylamide gel electrophoresis and stained with ethidium bromide.

Results

Epigenetic changes in the NET gene in panic disorder

Direct sequence analysis of the promoter region of the *NET* gene in the panic disorder patients revealed no divergence from previously published sequences.

Genomic DNA methylation detection DNA methylation involves the covalent addition of a methyl group to cytosine of CpG dinucleotide pairs. The *NET* gene promoter region is extraordinarily rich in CpG islands and so is a prime target for DNA methylation. Methylation-specific PCR demonstrated increased methylation of the promoter region of the *NET* gene in nine of 24 patients with panic disorder, but in no healthy subjects (Fig. 3), suggesting that *NET* gene silencing possibly underlies the impairment of *NET* function phenotype we describe in panic disorder.

MeCP2 enrichment on the hypermethylated NET promoter in panic disorder

The mechanisms underlying repression of gene expression following on promoter region DNA hypermethylation have recently been elucidated for MeCP2, the best characterized member of the methyl-CpG binding domain proteins (El-Osta and Wolffe, 2000; Harikrishnan *et al.,* 2005). To test for MeCP2 enrichment on the *NET* gene we employed chromatin immunoprecipitation (ChIP). The soluble chromatin fractions were prepared in two representative healthy subjects and four panic disorder patients and immunoprecipitated with αMeCP2 antibody. In the healthy subjects MeCP2 binding to *NET* gene DNA was not demonstrable. MeCP2 was clearly enriched on the promoter region of the *NET* gene in all four panic disorder patients, suggesting that methylation-mediated silencing accounts for the impaired *NET* function in panic disorder.

Why panic disorder patients have heavy methylation of *NET* gene promoter region DNA remains unknown. Unlike in Fragile X disease, where DNA hypermethylation is due to expansion and hypermethylation of CpG repeats, sequencing of promoter region DNA in POTS disclosed no similar abnormalities, although we cannot rule out changes in other elements such as distant enhancers.

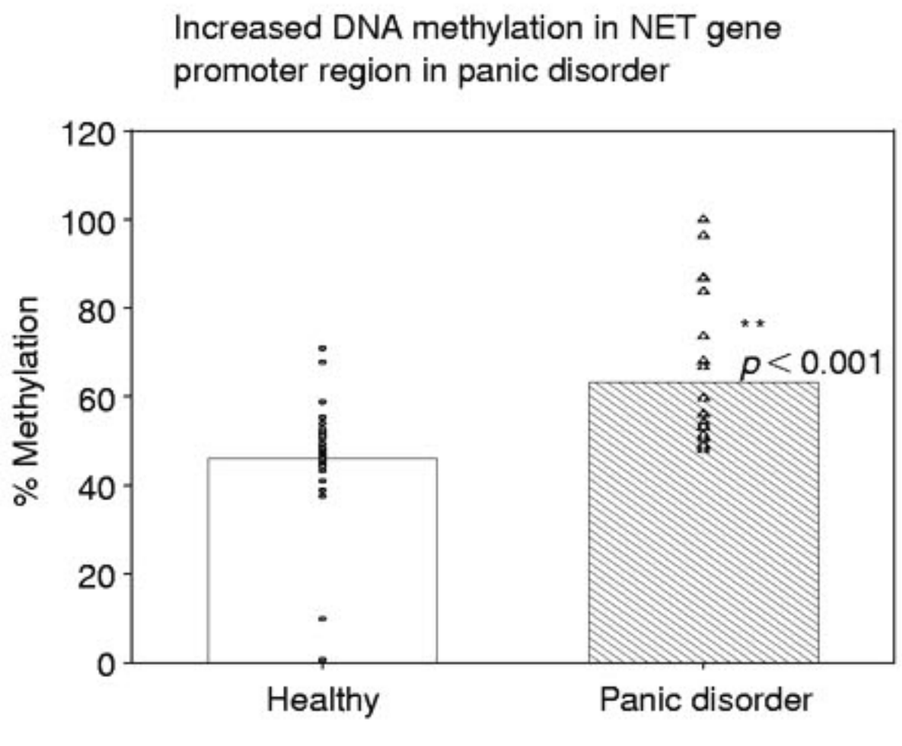

Figure 3 Although there was overlap in the distributions, the percentage of methylation of DNA in the selected CpG rich promoter region in the NET gene was higher in panic disorder. Chromatin immunoprecipitation methodology demonstrated binding of the gene silencing transcription factor, MeCP2, to NET gene promoter region DNA (not shown in figure) in four of the panic disorder patients with the highest level of DNA methylation

NET gene silencing in panic disorder?

DNA methylation is a major mechanism of epigenetic silencing of genes. Repression of transcription by gene promoter region methylation is known to be important in human development and the development of malignancies. It has not been possible to date to definitively demonstrate the presence of *NET* gene silencing in panic disorder patients based on the documentation of reduced levels of *NET* gene mRNA. In both healthy subjects and panic patients we tested for but could not detect *NET* gene mRNA in leucocytes of blood buffy coat. Obtaining a suitable tissue source for *NET* gene mRNA assay in POTS patients will be difficult. Even tissues with a rich sympathetic innervation, such as small veins on the dorsum of the hand, which we have biopsied for sympathetic nerve neurochemical analyses in the past, may not be suitable. The terminal extensions of accessible sympathetic nerves in general are so far distant from the cell bodies in the sympathetic ganglia that distal flow of mRNA to the biopsy site is unlikely.

Consequences of impairment of neuronal reuptake of noradrenaline in panic disorder

Does faulty noradrenaline reuptake by sympathetic nerves 'cause' panic disorder?

Symptom-generating processes in the heart can lead to the development of panic disorder. It has been known for some time that the presence of abnormalities of heart rhythm, in particular supraventricular tachycardia, can antecede and in fact cause panic disorder (Lessmeier *et al.*, 1997). More recently, a very high rate of development of panic disorder has been observed in patients with implanted cardiac defibrillators (Godemann *et al.*, 2001), which are prone to discharge in error. Factors at play here are the presence of cardiac symptoms and the enhanced vigilance which accompanies them.

The impaired neuronal noradrenaline reuptake phenotype would be expected to magnify sympathetically mediated responses, particularly emotionally driven responses in the heart where noradrenaline inactivation is so dependent on neuronal reuptake (Eisenhofer *et al.*, 1996), perhaps causing a similar sensitization to cardiac symptoms and predisposing to the development of panic disorder. The panic disorder patients with most evident impairment of cardiac noradrenaline reuptake did fit into the 'cardiac panicker' diagnostic designation (Alvarenga *et al.*, 2006), where cardiovascular symptoms are especially prominent. We noted a relationship between impaired neuronal noradrenaline reuptake in the heart and anxiety 'sensitivity' (Taylor and Cox, 1998), (a psychometric measure of perceptions of enhanced and threatening cardiac symptoms leading to a fear of autonomic arousal) in panic disorder, suggesting that augmentation of sympathetic nerve firing in the heart by an impairment of neuronal noradrenaline reuptake was responsible for high anxiety sensitivity scores.

Does faulty noradrenaline reuptake by sympathetic nerves increase cardiac risk?

Reuptake of noradrenaline into sympathetic nerves after its release terminates the neural signal. A fault in transmitter inactivation augments the effects of sympathetic nerve traffic. For the sympathetic nerves of the heart approximately 95% of released noradrenaline is recaptured (Eisenhofer *et al.*, 1996), so that the heart is more sensitive than all other organs to impediments in transmitter reuptake. An abnormality in neuronal noradrenaline reuptake, through causing high concentrations of the sympathetic neurotransmitter in the synaptic space, would sensitize the heart to arrhythmia development during episodes of intense sympathetic activation, such as those occurring during panic attacks (Fig. 2).

Cardiovascular disorders co-morbid with panic disorder

The prevalence of essential hypertension (Davies *et al.*, 1999) and POTS (Goldstein *et al.*, 2002) is increased in patients with panic disorder. The pathophysiology of each disorder has unique elements, but the uniting link appears to be the phenotype of impaired neuronal reuptake of noradrenaline, which is common to all.

Essential hypertension

Essential hypertension is commonly 'neurogenic', initiated and sustained by the sympathetic nervous system (Grassi *et al.*, 1998; Esler, 2004). Activation of central sympathetic outflow has been well documented using microneurography, but a second neural mechanism, reduced reuptake of the sympathetic neurotransmitter,

noradrenaline is also operative. In work spanning two decades, with papers from 1981–2001 (Esler *et al.*, 1981; Rumantir *et al.*, 2000), using isotope dilution methodology we have consistently documented a phenotype of impaired neuronal reuptake of noradrenaline in essential hypertension. There are no identified loss of function *NET* gene coding region mutations in hypertension, and the mechanisms that underlie *NET* silencing have remained elusive.

As for panic disorder, we have recently noted epigenetic changes in the *NET* promoter which are functionally linked with the phenotypic disposition of hypertensive patients (Guo *et al.*, 2005), detecting *NET* promoter DNA hypermethylation in the majority of hypertensive individuals tested (26 of 33), but not in healthy subjects. This finding followed from repeat testing on the index patient with the most extreme reduction in noradrenaline reuptake from the 1981 paper. In 2004 he was found to have unremarkable *NET* gene coding region sequences but promoter region hypermethylation (Guo *et al.*, 2005).

Postural tachycardia syndrome

The postural tachycardia syndrome (also known as autonomic intolerance) has only recently come to international medical attention (Shannon *et al.*, 2000; Goldstein *et al.*, 2002). The syndrome has multiple symptoms, chief among which are symptomatic tachycardia, weakness and recurrent blackouts while standing. There is typically minimal fall in blood pressure on standing, despite the tendency to faint. Although there has been recent publication of numerous papers in high profile medical journals, this disabling condition is still not well known to the medical profession, and receives little attention as yet in textbooks of medicine. The defining characteristic of POTS is orthostatic intolerance accompanied by an excessive reflex activation of the sympathetic nervous system on standing, with the excessive reflex rise in heart rate providing the cornerstone of diagnosis (Shannon *et al.*, 2000; Goldstein *et al.*, 2002) The symptoms and ongoing disability appear to primarily result from this supernormal reflex sympathetic activation with standing.

The causes of POTS remain uncertain and are perhaps multiple, but excessive pooling of blood in the veins of the legs on standing (Goldstein *et al.*, 2002) and impairment of the neuronal reuptake of norepinephrine (Shannon *et al.*, 2000; Goldstein *et al.*, 2002) seem to be of prime importance. In one family kindred the cause of the latter has been traced to a point mutation in the coding region of the *NET* gene resulting in the production of a dysfunctional transporter protein (Shannon *et al.*, 2000), but in the remainder of POTS patients, despite extensive testing, no similar loss of function mutation has been identified and the cause of the phenotype of impaired *NET* activity has remained unknown.

In our recent testing for an epigenetic mechanism, we documented very heavy (100%) methylation of *NET* gene promoter DNA in the majority of POTS patients tested (unpublished observations), accompanied by high tires of bound gene silencing transcription factor (MeCP2), which we believe underlies the impairment of *NET* function phenotype in POTS and is a causal factor in the disorder.

What are the unique mediating neural pathophysiologies?

As panic disorder, essential hypertension and POTS all appear to have *NET* gene silencing, why are they just commonly comorbidities rather than being invariably clinically totally concordant? Perhaps the clinical expression of each disease depends on an associated pathophysiology which is unique to each?

In *panic disorder,* sensitization of the heart by faulty neuronal reuptake of noradrenaline, such as to lead to cardiac symptoms, may be a common and sufficient mechanism. In principle, the mechanism is seen as being similar to that with supraventricular tachycardia (Lessmeier *et al.*, 1997) and implantable cardiac defibrillators (Godemann *et al.*, 2001) described earlier. In POTS, where *NET* gene and postural venous pooling abnormalities coexist to cause symptomatic tachycardia primarily related to standing, perhaps a transition to panic disorder occurs, by an analogous sensitization to heart symptoms. The neural prerequisite for essential hypertension might be the well documented persistent activation of sympathetic outflow (Grassi *et al.*, 1998; Esler, 2004), augmented by the neuronal noradrenaline reuptake defect (Esler *et al.*, 1981; Rumantir *et al.*, 2000). In POTS and panic disorder such a persisting increase in sympathetic nerve firing rates is absent.

Brain monoamine neurotransmitter turnover in panic disorder

The brain neurotransmitter changes characterizing panic disorder are uncertain. The noradrenergic neurons of the locus coeruleus, demonstrated in experimental animals to be involved in mediating stress responses and rostral serotonergic projections from the brainstem raphe nuclei have both been implicated (Singewald *et al.*, 1997)

We used direct methodology to quantify brain norepinephrine and serotonin turnover in untreated patients with panic disorder, based on the overflow into the internal jugular veins of norepinephrine and its major lipophilic metabolites, 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3,4-dihydroxyphenylglycol (DHPG) and of the principal brain serotonin metabolite, 5-HIAA (Lambert *et al.*, 2002; Esler *et al.*, 2004). A total of 27 patients who were either never treated or had received no medication in the preceding 3 months were studied.

Brain norepinephrine turnover, estimated with bilateral jugular venous sampling, or from doubling the unilateral jugular venous overflow of norepinephrine, MHPG and DHPG (findings in volunteers having bilateral sampling justified this adjustment), was unremarkable in panic disorder. With the assistance of the Melbourne Bureau of Meteorology we have previously demonstrated an influence of season and sunlight on brain serotonin release in humans (Lambert *et al.*, 2002), so the brain serotonin turnover in panic disorder patients was expressed in relation to seasons, against this database (Esler *et al.*, 2004). Overall, across the year, brain serotonin turnover, estimated from the jugular venous overflow of 5-HIAA, was increased approximately seven-fold in panic

disorder (Esler *et al*., 2004). To this point jugular venous sampling has not been performed during a panic attack.

It is probable that the pronounced increase in serotonin turnover noted at rest in panic sufferers, in the absence of a panic attack, represents the underlying neurochemical substrate for the disorder. Whether this brain serotonergic activation is a prime mover, or consequential on other primary causes of panic disorder, including cardiac sensitization by faulty neuronal noradrenaline reuptake leading to cardiac symptoms and the enhanced vigilance which accompanies them, is unclear at present.

Note

*Professor Jeff Richards died on 5 April 2005.

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