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NOREPINEPHRINE TRANSPORTER-DEFICIENT MICE RESPOND TO ANXIETY PRODUCING AND FEARFUL ENVIRONMENTS WITH BRADYCARDIA AND HYPOTENSION

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Abstract—The study of anxiety and fear involves complex interrelationships between psychiatry and the autonomic nervous system. Altered noradrenergic signaling is linked to certain types of depression and anxiety disorders, and treatment often includes specific transporter blockade. The norepinephrine transporter is crucial in limiting catecholaminergic signaling. Norepinephrine transporter-deficient mice have increased circulating catecholamines and elevated heart rate and blood pressure. We hypothesized, therefore, that reduced norepinephrine clearance would heighten the autonomic cardiovascular response to anxiety and fear. In separate experiments, norepinephrine transporter-deficient (norepinephrine transporter$^{-/-}$) mice underwent tactile startle and trace fear conditioning to measure hemodynamic responses. A dramatic tachycardia was observed in norepinephrine transporter$^{-/-}$ mice compared with controls following both airpuff or footshock stimuli, and pressure changes were also greater. Interestingly, in contrast to normally elevated home cage levels in norepinephrine transporter-deficient mice, prestimulus heart rate and blood pressure were actually higher in norepinephrine transporter$^{+/+}$ animals throughout behavioral testing. Upon placement in the behavioral chamber, norepinephrine transporter-deficient mice demonstrated a notable bradycardia and depressor effect that was more pronounced in females. Power spectral analysis indicated an increase in low frequency oscillations of heart rate variability; in mice, suggesting increased parasympathetic tone. Finally, norepinephrine transporter$^{-/-}$ mice exhibited sexual dimorphism in freeze behavior, which was greatest in females. Therefore, while reduced catecholamine clearance amplifies immediate cardiovascular responses to anxiety- or fear-inducing stimuli in norepinephrine transporter$^{-/-}$ mice, norepinephrine transporter deficiency apparently prevents protracted hemodynamic escalation in a fearful environment. Conceivably, chronic norepinephrine transporter blockade with transporter-specific drugs might attenuate recognition of autonomic and somatic distress signals in individuals with anxiety disorders, possibly lessening their behavioral reactivity, and reducing the cardiovascular risk factors associated with persistent emotional arousal.

Key words: norepinephrine, transporter, blood pressure, heart rate, behavior, fear.

The norepinephrine transporter (NET) regulates sympathetic tone by removal of residual synaptic catecholamines, including norepinephrine (NE) and dopamine (DA), and to some extent, epinephrine (EPI). Peripherally, NET limits adrenoreceptor-mediated signaling in smooth and cardiac muscle, glandular structures, parenchymal organs and cutaneous structures. Centrally, NET is localized in brainstem nuclei, amygdala, cerebellum, cortex, hippocampus, hypothalamus, thalamus, and the bed nucleus of the stria terminalis (Schroeter et al., 2000); sectors of cardiovascular regulation, sensorimotor processing, emotion, and memory.

Autonomic regulation of basal circulation is normally a balance of sympathetic and parasympathetic tone to accommodate activity and positioning. Within the baroreflex arc of the brainstem, activation of one branch of the autonomic nervous system (ANS) is typically counterbalanced by inhibition of the other (Chiu et al., 2003). Emotions originating in higher brain centers such as sadness, pleasure, anxiety or fear, can override basal cardiovascular control, producing increases or decreases in heart rate (HR) and blood pressure (BP). For example, sudden exposure to stressful or fear-inducing stimuli can result in the rapid onset of hypotension and bradycardia, causing fainting in humans (Shen et al., 2000).

Altered noradrenergic signaling is linked to certain types of depression (Hadley et al., 1995; Anand and Charney, 2000) and anxiety disorders (Aston-Jones et al., 1994; Bremner et al., 1996; Biederman and Spender, 2000; Van Bockstaele et al., 2001). Treatment often incorporates NET blockers (Morilak and Frazer, 2004) like re-

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Abbreviations: AUC, area under the curve; BP, blood pressure; BPV, blood pressure variability; DA, dopamine; GAD, generalized anxiety disorder; HR, heart rate; HRV, heart rate variability; HF, high frequency; KO, knockout mouse; LC, locus coeruleus; LF, low frequency; NE, norepinephrine; NET, norepinephrine transporter; NTS, nucleus tractus solitarii; PAG, periaqueductal gray area; PSD, power spectral density; S.E.M., standard error of the mean; WT, wild-type mouse.

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boxetine or atomoxetine, which transiently increase HR and BP while decreasing sympathetic tone (Schroeder et al., 2002; Tank et al., 2003). The long-term modulatory effects of NET blockade on central processing of anxiety-producing and fearful stimuli, however, are not fully understood, nor is the impact of such treatment on the physiologic alerting signals that accompany emotional distress.

The autonomic response to psychological stress prepares the organism for "fight-or-flight" (Ziegler, 2004), which has recently been reconceptualized as "freeze, flight, fight, fright and (in humans) faint" (Bracha, 2004). A perceived threat can induce either vigilance or defensive behavior and is manifest by a state of fear or anxiety (Lang et al., 2000). Fearful vigilance, exhibited in rodents as freezing behavior, allows orientation to surroundings and attention to the source of threat. It is accompanied by decreased BP and HR (Lang et al., 2000). A defensive response and sympathoadrenal activation may follow, signaling release of epinephrine and NE, corticotropin-releasing factor, vasopressin and glucocorticoids (Van Bockstaele et al., 2001; Carrasco and Van de Kar, 2003). Defensive behavior, including flight and fight, is associated with increased BP and tachycardia, enhanced cardiac output and respiration, increased cerebral perfusion, potentiated somatic reflexes and redistribution of blood flow to increase limb circulation (Bandler et al., 2000; Lang et al., 2000; Maren, 2001; Carrasco and Van de Kar, 2003). These responses were undoubtedly beneficial to mammalian survival, but chronic exposure to even low-level stressors in modern everyday life elicits detrimental cardiovascular effects, increasing the risk for hypertension, arrhythmias, cardiomyopathy and myocardial infarction (Folkow, 1987; McEwen, 1998; Esler et al., 2004).

Anxiety is a state of emotional distress elicited by cues signaling potential—not immediate—danger. Like fear, it is accompanied by physiological hyperarousal (Morilak and Frazer, 2004), but behavior varies (Lang et al., 2000; Maren, 2001) and anxiety disorders in humans are often resistant to extinction (Maren, 2001). Anxiety disorders including panic, phobias, obsessive–compulsive disorder, and generalized anxiety disorder (GAD) are often complicated by concomitant psychopathologies, particularly depression (Sramek et al., 2002). Women are more likely to develop anxiety-based maladies than men, including post-traumatic stress disorder (11.3% vs. 6.0%), panic disorder (5.0% vs. 2.0%), simple phobia (15.7% vs. 6.7%), and GAD (6.6% vs. 3.6%) (Piggott, 2003). Furthermore, anxiety disorders have been linked to an increased susceptibility for depression in both sexes, and the risk for major depression in women is nearly twice that of men (Kendler et al., 2003; Piggott, 2003).

Panic disorder carries with it an increased risk for sudden death and cardiovascular mortality (Kawachi et al., 1994), and has been linked to decreased heart rate variability (HRV) and vagal tone (Kawachi et al., 1995). HR and BP increase dramatically in humans during a panic attack due to sympathoadrenal discharge. Epinephrine release from the adrenal medulla and sympathetic nerve endings increases two to six times over resting levels, and is greatest in the heart (Wilkinson et al., 1998).

Autonomic testing in humans, by necessity, is a somewhat passive process carried out under isolated conditions with minimal interference from exogenous stimuli. Normal living is rarely so quiescent, however, and environmental and emotional stimuli frequently enter the picture. Animal models permit study of the autonomic and behavioral effects of anxiety- and fear-inducing stimuli under similarly controlled conditions (Taylor and Printz, 1996; Lang et al., 2000; Muller and Keck, 2002) that provide a slightly more holistic outcome. The startle response, for example, can be elicited in vertebrates by tactile, acoustic or vestibular stimulation, and is used to dissect central mechanisms of sensorimotor integration and anxiety-related behaviors (Koch, 1999). We used tactile startle to introduce rapid hemodynamic perturbations in conscious mice and examine the cardiovascular effects of NET-deficiency under anxiety-inducing conditions.

To produce fearful conditions, we used classic trace fear conditioning in which a non-threatening stimulus is followed a brief time later by an aversive stimulus (Maren, 2001). After conditioning, introduction of the innocuous stimulus alone will bring forth a duplication of the emotional and physiologic responses as if the noxious stimulus was also presented. Because the stimulus is remote with no discernible means of escape, the behavioral response of mammals such as rodents is often "freezing", a passive coping mechanism accompanied by hypotension, bradycardia, and increased respiration (Lang et al., 2000; Carrasco and Van de Kar, 2003).

Behavioral phenotyping of $NET^{-/-}$ mice to date has been limited to males, and includes prolonged escape attempts in tail suspension and forced swim tests (Xu et al., 2000). Social distress increased defensive behaviors in both $NET^{+/+}$ and $NET^{-/-}$ males with repeated intruder contact, however, subsequent exposures to dominant mice revealed divergent behaviors (Haller et al., 2002). $NET^{+/+}$ animals increased escape attempts while the prevalent reaction in NET-deficient mice was freezing. Later, defeat-stressed $NET^{-/-}$ animals struggled more than $NET^{+/+}$ mice in a behavioral despair model. Together, these data suggest less susceptibility to depression-like behavior in naïve, non-stressed $NET^{-/-}$ males, and introduce the possibility of decreased defensive behavior in chronic socially challenging situations.

We recently reported elevated BP and HR in conscious undisturbed $NET^{-/-}$ mice at rest and with activity in the home cage setting (Keller et al., 2004). Continuous telemetric recordings evaluated over 12-h light/dark periods demonstrated that BP and HR are only slightly increased in resting NET-deficient mice in comparison to controls. This is consistent with normal physiology, since sympathetic outflow is dampened during rest and sleep (Ziegler, 2004). Furthermore, reduced basal sympathetic tone is apparently augmented in $NET^{-/-}$ mice, and we have demonstrated decreased renal sympathetic nerve activity in anesthetized animals (Diedrich et al., submitted for publication), supporting the premise of enhanced central sympathoinhibi-