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PERSPECTIVE

Center Stage for the Serotonin Transporter: A Gain-of-Function Polymorphism in Persons with Obsessive-Compulsive Disorder

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Monoamine transporters selective for dopamine, norepinephrine, and serotonin (5-HT) are presynaptic plasma membrane proteins responsible for the reuptake of released transmitters from the synaptic cleft into nerve terminals. This process not only limits the intensity and the duration of monoamines at pre- and postsynaptic receptors but is also the primary mechanism by which monoamine neurons maintain a transmitter pool available for subsequent release. Pharmacological approaches, and more recently gene targeting technologies, have revealed the profound neurochemical and behavioral consequences of disrupting the function of each of these transporters in mice (Gainetdinov and Caron, 2003). Clinically, monoamine transporters are important pharmacological targets for many therapeutic agents currently used to treat a variety of human conditions, including depression, obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), and eating disorders. In addition, psychostimulants, including cocaine, amphetamine, and (+)-3,4-methylenedioxymethamphetamine, exhibit their rewarding and reinforcing actions primarily by acting at monoamine transporters (Amara and Sonders, 1998).

Not surprisingly, monoamine transporters have been the subject of intensive investigation by both basic and clinical researchers. Over the past few years, we have witnessed a true explosion of information dealing with the functional and regulatory aspects of monoamine transporters using both in vitro and in vivo approaches (for review, see Torres et al., 2003b). These studies have examined the functional relevance of transporter trafficking, modification of transporter function by intracellular signaling, mechanisms of assembly and oligomerization, interaction with regulatory proteins, transporter-associated currents, structure-function analysis, brain imaging analysis with selective ligands, and genetic ablation of transporter genes in vivo.

Given the importance of transporters in regulating monoamine homeostasis, research efforts have recently been focused on the search for polymorphic variations that might help explain the pathophysiology of certain human conditions; alternatively, these variants could represent important factors conferring disease susceptibility. In the case of the dopamine transporter, a variable number of tandem repeat polymorphism has been identified in the 3′-untranslated region of the transporter. Alleles with 10 copies of a 40-base repeat unit have been reported in several populations of patients with ADHD (DiMaio et al., 2003). The transcriptional control region of the 5-HT transporter (SERT) contains a functional polymorphism (5-HTTLPR) consisting of an insertion (long allele, l) or a deletion (short allele, s) of 44 bases. These polymorphic variants are associated with differences in SERT gene expression and function that have been proposed to be linked with anxiety, depression, ADHD, and other mood-related disorders (Heils et al., 1997). However, the data thus far have not been totally conclusive, motivating further research.

In this issue of *Molecular Pharmacology*, Kilic and colleagues describe the functional consequences of a single nucleotide polymorphism in the coding region of the human SERT (Kilic et al., 2003). An isoleucine-to-valine substitution (I425V) in transmembrane domain 8 of the transporter was found as a rarely occurring variant of the human SERT. Analysis of the mutant transporter in heterologous cells revealed an increase of ~2-fold in uptake activity. This effect was caused by an increase in both the maximal uptake activity (higher V_{max}) and the affinity of 5-HT for the transporter (lower K_m) compared with the wild-type transporter. Changes in the levels of transporter expression at the plasma membrane were excluded as the cause for the increase in uptake activity. Thus, the observed gain-of-function phenotype is consistent with a change in the intrinsic properties of the mutated transporter rather than with changes in trafficking and/or transporter turnover. This residue is conserved in all SERT species; however, other members of the

ABBREVIATIONS: 5-HT, serotonin; SERT, serotonin transporter; ADHD, attention deficit hyperactivity disorder; OCD obsessive-compulsive disorder.

monoamine transporter family already have a valine residue at this position.

Interestingly, the mutant transporter was insensitive to up-regulation by a nitric oxide-dependent pathway under conditions in which wild-type SERT activity is increased by activation of this pathway. This observation led the authors to suggest the intriguing possibility that the mutation might mimic the effects of nitric oxide on the wild-type transporter. Although the effect of increasing NO levels on SERT through the activation of adenosine receptors has been documented in basophilic leukemia cells (Miller and Hoffman, 1994), the physiological consequences of this mode of regulation in the brain are not understood (Asano et al., 1997). However, the findings by Kilic and colleagues highlight the exciting possibility that additional differences may exist in the phenotype of the wild-type and mutant transporters. For instance, recent evidence suggests that monoamine transporters function as oligomeric complexes (Kilic and Rudnick, 2000; Hastrup et al., 2001; Sorkina et al., 2003; Torres et al., 2003a). In that context, naturally occurring gain- or loss-of-function transporter mutants should display more than simple additive (gain of function) or subtractive (loss of function) effects on transporter activity. Additional differences might include changes in phosphorylation levels, the ability to interact with regulatory proteins and, potentially, the differential ability of inhibitors to block transporter uptake activity.

What is the physiological significance of this novel SERT variant? The discovery of the I425V mutation originated in a study by Ozaki and et al. (2003). These authors genotyped a population of 383 neuropsychiatric patients and healthy control subjects. The I425V variant was detected in two unrelated patients affected with OCD and other psychiatric disorders. Subsequent analysis of the families of these patients with OCD revealed that six of the seven persons who were heterozygous for the I425V allele had been previously diagnosed with OCD as well as several other psychiatric conditions, including anorexia nervosa, Asperger's syndrome, social phobia, and alcohol abuse.

OCD is a severe mental condition characterized by repetitive thoughts (obsessions) and repetitive actions (compulsions) accompanied by a marked impairment in life quality. Although the pathophysiology of this disease is poorly understood, effective treatment can be achieved in approximately half of patients with OCD by the use of selective 5-HT reuptake inhibitors. The effectiveness of serotonergic medications in the treatment of patients with OCD has lead to the hypothesis of a serotonergic dysfunction in the pathophysiology of OCD. Consequently, the SERT gene has received considerable attention as a candidate gene for OCD.

To our knowledge, this report is the first to identify a naturally occurring coding mutation in persons with a psychiatric condition for any of the three monoamine transporters. In addition to the presence of the I425V mutation, the two original probands and their two siblings were all homozygous for the long allele of the 5-HTTLPR polymorphism. Because the long allele polymorphic variant is associated with an increased expression and function, the combination of these two polymorphic variants should produce a further increase in transporter activity and perhaps additional phenotypic differences. In the study by Ozaki et al. (2003), most patients with OCD did not carry the I425V mutation. However, those identified with the I425V SERT variant were found to be resistant to treatment with selective 5-HT reuptake inhibitors. As pointed out by the authors, it is possible that the mutation in transmembrane domain 8 of SERT alone or in combination with the promoter polymorphism may confer resistance to the clinical effects of SERT inhibitors in these patients. These findings are of tremendous interest. However, because the SERT variant was found in low frequency and a relatively low number of individuals were genotyped, examination of larger and ethnically diverse populations will be required to determine the true incidence and clinical relevance of this genetic variant for OCD.

The search for polymorphic forms of monoamine transporter genes should be a fertile approach. Single-nucleotide polymorphisms have already been described for the human dopamine transporter and the human norepinephrine transporter (Mazei and Blakely, 2002; Hahn and Blakely, 2002; Lin and Uhl, 2003) but none of these variants has been shown to change transporter function in a manner that could contribute to a neuropsychiatric disease. When considering other diseases, however, Shannon et al. (1999) have identified a loss-of-function mutation in the coding region of the norepinephrine transporter in a patient suffering from orthostatic intolerance and tachycardia. The extent of the variability of SERT and other monoamine transporters in healthy and nonhealthy populations and their potential contributions to human conditions is just beginning.

At the structural and functional levels, these findings may provide insights into the mechanism of transport function. Hundreds of engineered mutations have been described in the monoamine transporter gene family. Kilic and colleagues (2003) have now characterized the first gain-of-function mutation in one of these genes. It will be of great interest to establish a full pharmacological profile of this SERT variant and determine the mechanistic bases for its enhanced uptake activity. Ultimately, "humanizing" a mouse with this SERT variant might provide a useful model to examine the consequences of such mutation on monoamine homeostasis.

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