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How the Monoamine Transporter Garden Grows

Arnold E. Ruoho

Department of Pharmacology, University of Wisconsin Medical School, Madison, WI, 53706 USA.

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Running Title: Human Norepinephrine (hNET) Transporter Polymorphisms

Corresponding author:

Arnold E. Ruoho
Department of Pharmacology – MS#014951
University of Wisconsin Medical School
1300 University Avenue
Room 4775 MSC
Madison, WI 53706-1532
Voice: (608) 263-5382
Fax: (608) 262-1257
Email: aeruoho@wisc.edu

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Abstract

In this issue of Molecular Pharmacology, Hahn, Mazei-Robison and Blakely present a study of previously uncharacterized single amino acid variants of the human norepinephrine transporter (hNET). Intracellular trafficking, surface expression, transport properties, interaction with antagonists and regulation by a protein kinase C (PKC) linked regulatory pathway were studied by heterologous expression in COS-7 cells. In recent years, there has been increasing focus on the natural variations and roles of nonsynonymous single nucleotide polymorphisms (SNPs) in human membrane transporter genes (and their protein products) in human disease. As this information is assimilated and understood at the molecular and genetic level, the relationship between transporter pharmacogenomics and therapeutics in the age of individualized medicine will be greatly impacted.

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Over the past 30 years, the time between basic science discoveries and the application of new information to the treatment of human disease has become increasingly compressed. For example, consider the translational relationship between the purification and characterization of the nicotinic neuromuscular junction acetylcholine receptor (Berman and Patrick, 1980; Lindstrom, 2002; Lindstrom, 2000; Patrick and Lindstrom, 1973) and the application of this knowledge to therapeutic treatment regimens for myasthenia gravis. Similarly, consider the discoveries and exhaustive characterization over a few short years related to drug metabolism and exemplified in the P450 (CYP) field. The dramatic effects that this information has provided to our understanding of, not only drug pharmacodynamics in humans, but also the ethnic genetic variations in world populations are truly profound (Cascorbi, 2003; Schwarz, 2003). Knowledge of the effects of CYP polymorphisms in drug metabolism is influencing the approaches by which physicians treat human diseases (Dahl, 2002; Kirchheiner and Brockmoller, 2005; Roots et al., 2004; Tempfer et al., 2004). New drugs are now synthesized and screened in the pharmaceutical industry to meet strict requirements dictated by known drug metabolic pathways (Rodrigues and Rushmore, 2002).

Many researchers have been engaged in exciting and highly significant experiments identifying and understanding the consequences of single nucleotide polymorphisms (SNPs) and other genetically transmitted alterations in neurotransmitter receptors (Kirstein and Insel, 2004; Liggett, 2004; Michel and Insel, 2003; Small et al.,

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2003), ion channels (Anson et al., 2004; Furutani et al., 1999; Zhou et al., 1999) and enzymes important in cellular and organismal homeostasis.

Membrane transporters play a critical role in maintaining physiological balance in the biological kingdom. Both plasma membrane and intracellular membrane transporters compartmentalize metabolic processes including the synthesis and sequestration of neurotransmitters and the elimination of toxic waste products and xenobiotics. Many natural variations resulting in single nucleotide polymorphisms (SNPs) in transporter genes which are drug targets have been identified (Leabman et al., 2003). These transporters include the ABC superfamily (e.g. ATP binding cussette transporters, e.g., MDRI) which remove xenobiotics from cells and SLC (solute carrier) superfamily transporters (e.g. OCT1, DAT, NET, VMAT1) which sequester neurotransmitters and small molecules into and within cells.

The monoamine transporters for norepinephrine (NE), dopamine (DA) and serotonin (5-HT) belong to the SLC6A family of Na^+/Cl^- dependent transporters. Several SNPs for the human norepinephrine transporter (hNET), the human dopamine transporter (hDAT) and the human serotonin transporter (hSERT) have been reported and many have been characterized at the cellular level (Hahn and Blakely, 2002a). Some transporter variants that relate to human phenotypes have been successfully linked within families (Hahn and Blakely, 2002a).

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In this issue of Molecular Pharmacology, the paper entitled "Single Nucleotide Polymorphisms in the Human Norepinephrine Transporter Gene Impact Expression, Trafficking, Antidepressant Interaction and Protein Kinase C Regulation" characterizes several properties of the norepinephrine transporter (NET) with regard to amino acid variants that have not been previously characterized in human populations that include cardiovascular phenotypes. Hahn, Mazei-Robison and Blakely (2005) have significantly enhanced our knowledge of the characterization of hNET SNPs that influence transporter function at the cellular level. These investigators previously identified a SNP of hNET (A457P) which is directly linked to an autonomic disorder, orthostatic intolerance (OI) (Hahn et al., 2003; Shannon et al., 2000). This condition is characterized by an elevated heart rate and increased plasma norepinephrine levels in family members. Since NET resequesters most of the NE released in the heart, this reuptake system in the sympathetic neurons serving the heart assumes a singular role compared to the brain where regulation between several neurotransmitter systems (including NE) is shared through interactive regulatory pathways (Hahn and Blakely, 2002a; Hahn and Blakely, 2002b).

Most of the hNET variants reported in the current Hahn et al study have also been identified in populations having cardiovascular phenotypes which include extremes of blood pressure or long QT syndrome (Halushka et al., 1999; Iwasa et al., 2001), but have not been previously characterized at the cellular level. Figure 1 shows the nonsynonymous SNPs that were characterized in this study (open circles) in the background of additional nonsynonymouns SNPs previously identified (closed circles). Expression levels of each SNP were assessed by western blots following transient

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transfection in COS-7 cells. Surface expression of the hNET variants was identified by sensitivity to surface biotinylation. Transporter function was measured by norepinephrine (NE) and dopamine (DA) uptake.

The hNET variants, V244I (TM4), V356L (TM7), N375S (ECL4) and K463R (TM9/ECL5) had little effect on hNET expression or transport of NE and DA. These data indicate that the relatively conservative amino acid substitutions at these positions are stably tolerated as further evidenced by comparison of monoamine transporter sequences from several species where various amino acid substitutions at these analogous positions are allowed.

On the other hand, several SNPs were demonstrated to result in unfavorable substitutions. The most dramatic substitution involved A369P (TM7/ECL4) which resulted in the total loss of surface expression of the mature 110 kDa transporter and loss of NE and DA transport of the surface expressed 54kDa molecules. Additionally, this hNET variant, as also previously reported for the A457P allele (Hahn et al., 2003) decreased total and surface levels of hNET demonstrating an important dominant negative suppression of wild-type transporter expression and function. The potential synergism of diminished wild type neurotransmitter transporter activity through dominant negative oligomerization in individuals carrying variant alleles is particularly significant and is likely to further exacerbate cardiovascular deficiencies.

Three additional striking ideas that were generated in the current study could have profound consequences: 1) The hNET F528C (TM11) allele showed a selective change in transport for NE over DA. This analogous region of the serotonin transporter (SERT) has been identified as extremely important for determining the potencies of substrates and

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antidepressants and similarly for functional activity of the dopamine transporter (DAT). The notion that variant alleles in the TM11 region (including hNET) could impact drug responses in these individuals is especially significant. 2) Additionally, as the authors correctly point out, variants in the F528 region of hNET could well alter the balance of monoamine neurotransmission in areas of the brain (e.g., the prefrontal cortex) where free DA is cleared by NET (Gresch et al., 1995) with resultant elevation of both NE and DA and significant global consequences following use of selective NET antagonists. 3) Finally, altered functional responses of hNET variants (either expressed separately or in concert with wild type hNET) to protein kinase C regulation raises additional significant concerns related to hNET activity when variant alleles are present. Further experimental work is needed to fully understand the consequence of SNPs on PKC mediated regulation of hNET. Certainly it is reasonable to conclude that PKC regulation of variant transporters will be affected during both the basal and regulated "state" (compare F528C to R121Q) but the complete elucidation of functional interactions and PKC regulation must await further experimental investigation.

Taken together, these data add significantly to our knowledge base regarding the properties of SNP in hNET. The authors do an excellent job of correlating in a broader sense the significance of these mutations with regard to function and regulation and the correlative changes that have been observed in other monoamine transporters such as SERT (Hahn and Blakely, 2002a; Kilic et al., 2003; Lesch et al., 1996). Eventually, a thorough data base will be needed to fully understand human conditions that are affected by SNP in transporters and other drug targets. Significantly, this process is already under development by the National Institutes of General Medical Sciences (NIGMS) through

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the Pharmacogenomics Network (PharmGKB.org) as a repository of pharmacologically relevant genes (Hewett et al. 2002; Klein et al., 2004). Several entries are currently present from the SLC transporter superfamily including the hNET SNP A457P which is linked to orthostatic intolerance. As additional relevant linkages of genotypes and phenotypes for transporters are discovered, this pharmacogenomic knowledge base will be invaluable.

Although there is a long and exciting journey ahead to further define the role of genetic polymorphisms in many drug targets, the transporter families are already well seeded and nourished. The substantial significance of the work represented in this paper and analogous experiments performed in many other laboratories are worthy of our notice and support. Individualized medicine, with a molecular mechanism basis, long a dream of many, is one step closer to reality.

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Figure 1: Schematic representation of hNET depicting the amino acid variants generated by nonsynonymous SNPs that have been identified. SNPs characterized in this study are in open circles and those previously identified are in closed circles. (Hahn et al., *Mol Pharmacol*, 2005).

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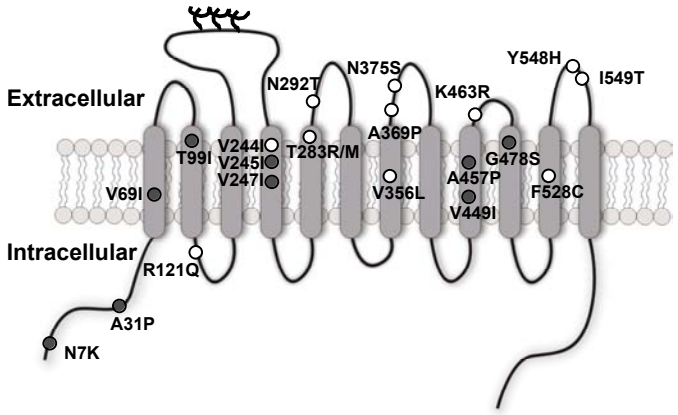
Figure 1


Extracellular
Intracellular
N292T
N375S
Y548H
I549T
K463R
A369P
T99I
V244I
V245I
V247I
T283R/M
G478S
A457P
V69I
V356L
F528C
V449I
R121Q
A31P
N7K