Editorial

Genotype and Vascular Phenotype Linked by Catecholamine Systems

David S. Goldstein, MD, PhD

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n this issue of Circulation, Fung and coworkers\(^1\) report associations between hand venous responses to the indirectly acting sympathomimetic amine tyramine and single nucleotide polymorphisms (SNPs) of genes encoding particular proteins related to the synthesis, release, reuptake, and metabolism of catecholamines. The importance of these associations lies in their illustrating how genotypic differences may contribute to phenotypic differences in circulatory functions in healthy adults via sympathetic neuroeffector mechanisms. More generally, identification of SNPs related to catecholamine systems may provide insights into the pathophysiology, diagnosis, and treatment of a variety of cardiovascular disorders.\(^2\)

To provide perspective about these findings, this editorial compares and contrasts tyramine-induced changes and sympathetic neuroeffector functions; discusses strengths and weaknesses of the dorsal hand vein model; emphasizes the potential of clinical catecholamine neurochemistry to link genotype with cardiovascular phenotype; and conveys a viewpoint on observational versus hypothesis-driven genotyping.

**Comparison and Contrast Between Tyramine-Induced Changes and Sympathetic Neuroeffector Functions**

Tyramine produces vasoconstriction via release of endogenous norepinephrine, the main neurotransmitter of the sympathetic nervous system mediating cardiovascular responses to stressors. The authors relied on the local vascular actions of tyramine as an indirectly acting sympathomimetic amine to draw inferences about sympathetic neuroeffector functions.

Mechanisms of tyramine-induced norepinephrine release differ in several respects from those of sympathetically mediated norepinephrine release. The Figure depicts some of these differences.

First, tyramine releases norepinephrine in a calcium-independent manner\(^3\) that is not exocytotic,\(^4\) in contrast to calcium-dependent exocytosis in response to sympathetic nerve stimulation. Tyramine displaces norepinephrine from storage vesicles, possibly by alkalinizing them.\(^5\) Because most of the vesicles would not be expected to be in communication with the extracellular fluid under resting conditions, most of the displaced norepinephrine would be expected to enter the cytoplasm rather than the extracellular fluid. Buildup of cytoplasmic norepinephrine would then lead to exit of norepinephrine into the extracellular fluid, such as via reverse transport through the cell membrane norepinephrine transporter (NET).\(^6\)\(^7\)

Second, because of the buildup of cytoplasmic norepinephrine, tyramine substantially augments oxidative deamination of norepinephrine, a process catalyzed by monoamine oxidase A in the outer mitochondrial membrane, converting norepinephrine to dihydroxyphenylglycol (DHPG), the main neuronal metabolite of norepinephrine. As a glycol, DHPG readily traverses the cell membrane to enter the extracellular fluid and plasma.\(^7\) In humans, plasma DHPG responses to intravenous tyramine therefore exceed plasma norepinephrine responses.\(^8\) In contrast, sympathetic stimulation results in similar absolute increments in plasma DHPG and norepinephrine levels, the increment in plasma DHPG in this setting reflecting reuptake of released norepinephrine into the cytoplasm via the NET.\(^9\)

Third, the NET operates as a high-affinity, low-capacity system. It is saturated at relatively low substrate concentrations. Thus, high plasma norepinephrine levels due to a pheochromocytoma interfere with cardiac uptake of the sympathoneural imaging agent 6-\([^{18}F]\)fluorodopamine.\(^10\) Tyramine might compete with endogenously released norepinephrine for reuptake into the cytoplasm via the NET, so that for a given amount of norepinephrine released from sympathetic nerves, a greater proportion would be delivered to adrenoceptors than during sympathetic stimulation.

Fourth, tyramine in sympathetic vesicles is converted to octopamine via hydroxylation catalyzed by dopamine-\(\beta\)-hydroxylase. The octopamine so produced may act as a false neurotransmitter.\(^11\)

Fifth, obtained statistical associations between SNPs and tyramine-induced vascular responses could reflect individual differences in vesicular storage or cytoplasmic metabolism of tyramine itself. This could apply to chromogranin B (CHGB), cytochrome b-561 (CYB561), and flavin-containing mono-oxygenase 3 (FMO3). The question therefore remains open about whether these SNPs are associated with the fate of endogenous catecholamines.

Given the differences between mechanisms of vascular responses to infused tyramine and to sympathetic stimulation, the observed statistical associations between tyramine-
induced vascular responses and frequencies of particular SNPs might not apply to sympathetic neuroeffector mechanisms in as straightforward a manner as the authors wished. In particular, the cell membrane and vesicular monoamine transporters are major determinants of sympathetic neuroeffector function. Hypofunctional polymorphisms of either transporter would be expected to be associated with decreased ability of tyramine to displace norepinephrine and therefore with decreased vasoconstrictor responses to tyramine. Because of the dependence of the study on the physiological consequences of displaced norepinephrine, the study was not designed in a manner that could distinguish altered uptake of tyramine from altered reuptake of norepinephrine. Decreased neuronal uptake might actually help to explain the seemingly paradoxical finding of less hand vein constriction in people with a positive family history of hypertension: Perhaps the individuals with a family history of hypertension had decreased cell membrane uptake of tyramine. There is support in the literature for the view that individuals with a family history of hypertension may have decreased NET activity.\textsuperscript{12,13}

If the overall purpose of the study were to understand better the potential roles of genetic polymorphisms for catecholamine-related genes in hypertension, it might have made more sense to use a test drug that increases sympathetic nerve traffic and augments exocytosis. $\alpha_2$-Adrenoceptor antagonists such as yohimbine increase norepinephrine release by these means,\textsuperscript{14} and among healthy humans, hypofunctional polymorphism of the $\alpha_2$-adrenoceptor is associated with augmented catecholamine and pressor responses to yohimbine.\textsuperscript{15} Because genetic polymorphisms for adrenoceptors or intracellular enzymes determining vascular effects of adrenoceptor occupation may influence responses to endogenously released norepinephrine, to take postsynaptic or extrasynaptic processes into account, it would seem important to assess local vasoconstrictor responses to directly administered norepinephrine.

**Strengths and Weaknesses of the Dorsal Hand Vein Model to Assess Sympathetic Neuroeffector Functions**

The investigators\textsuperscript{1} wished to assess effects of tyramine in a manner that would not be complicated or obscured by reflexive responses to the drug given systemically. Years ago, Miller and Streten,\textsuperscript{16} recognizing this advantage, measured dorsal hand vein responses to directly administered norepinephrine. Numerous other studies have used essentially the same approach, including at least 1 study involving vasoconstrictor responses to tyramine.\textsuperscript{17}

The dorsal hand vein model, however, entails potential limitations. It is generally accepted that sympathetic noradrenergic innervation of arterioles is more intense than that in the walls of large veins, and changes in arteriolar vascular resistance play a more important role in regulation of total peripheral resistance and therefore of blood pressure than do changes in diameter of cutaneous veins. It may therefore be difficult to extrapolate from findings in the dorsal hand vein to disorders involving increased total peripheral resistance, which characterizes the majority of cases of essential hypertension. This is one reason why studies of sympathetic neuroeffector function in humans have used the alternative model of brachial intra-arterial infusion of norepinephrine and other test drugs, including tyramine.\textsuperscript{18–21} The brachial arterial infusion model also enables assessment of effects of locally administered tyramine on levels of norepinephrine in the venous drainage of the arm.

**Clinical Catecholamine Neurochemistry to Link Genotype With Phenotype**

Fung et al\textsuperscript{1} relied on an indirect physiological measure (local vascular resistance) to indicate sympathoneural effects of infused tyramine. Local hemodynamic changes, however, may be related only indirectly and complexly to release of norepinephrine from sympathetic nerves. Applications of clinical catecholamine neurochemistry\textsuperscript{22} could have provided valuable insights about links between the SNPs of interest and the vascular phenotype.

Increments in plasma norepinephrine levels constitute a key dependent measure in the assessment of sympathetic neuroeffector function\textsuperscript{23} and have been used in studies of blood pressure responses to intravenous tyramine and forearm vascular responses to brachial arterial infusion of tyramine.\textsuperscript{21,24} Given access to a sensitive, specific radioenzymatic assay for catecholamines, the authors could have measured...
increments in plasma norepinephrine in ipsilateral versus contralateral antecubital venous plasma.

Because of the different sources and meanings of plasma levels of norepinephrine and DHPG, simultaneous measurements of these compounds provide a refined means to link genotype with phenotype. It is hoped that future studies will move beyond indirect physiological to more direct neurochemical correlates. For example, an observed association of a SNP for a monoxygenase with vascular responsiveness to tyramine could be followed up by measurement of DHPG-to-norepinephrine ratios in selected individuals.

The vascular responses to tyramine were highly variable across subjects. In a minority of subjects, tyramine actually produced local vasodilation, a phenomenon that local release of norepinephrine, a universal vasoconstrictor, could not easily explain. More detailed attention to intervening mechanisms might have helped to identify bases for this variability. For instance, conversion of tyramine in sympathetic vesicles to octopamine, a false neurotransmitter, might have limited vasoconstrictor responses to locally administered tyramine. Neurochemical data would also have helped in dealing with the thorny problem of contamination of dissolved tyramine by dopamine, infusion of which produces vasodilation. Vascular responses might also be influenced by local generalization of the vasodilator nitric oxide.

Local resistance responses to tyramine would be expected to be affected importantly by adrenoceptors both on the target smooth muscle cells and on sympathetic nerves. It is unfortunate that the present study did not include SNPs for adrenoceptors. For instance, SNPs of β2-adrenoceptors, associated with high plasma norepinephrine levels, have been reported to predict future renal damage and to be associated with indices of obesity or metabolic syndrome. Vasoconstrictor responses to intra-arterial tyramine have been found to be related inversely to directly recorded sympathetic nerve activity, suggesting a balance between norepinephrine delivery to and accessibility of vascular α-adrenoceptors in healthy humans.

**Observational Versus Hypothesis-Driven Genotyping**

Over the past decade or so, numerous reports have noted statistical associations between various cardiovascular abnormalities and SNPs. Peer-reviewed journals have recently become more demanding, asking not only for statistical associations but also for studies of functional consequences of SNPs. Thus, there is a movement from mainly descriptive to more physiologically meaningful information. A power of the approach used by Fung et al is that it is deductive (ie, hypothesis driven), in that the SNPs chosen for evaluation reflect processes known to contribute to sympathetic neuroeffector functions.

A weakness of such a deductive approach is that the SNPs chosen are based on hypotheses derived from a limited fund of knowledge. One cannot prove a negative, and therefore when statistical associations fail to be established, the possibility of a real relationship remains. The study might have been powered inadequately, the population might not have been stratified correctly, the dependent measures might have been too complexly or indirectly determined, the wrong SNPs might have been chosen, and so forth. The justification for the choice of SNPs evaluated in the study of Fung et al was high prevalence in the population; however, the most prevalent SNPs might contribute relatively weakly to sympathetic neuroeffector functions, whereas less common SNPs might contribute more to disease processes.

There are many proteins, perhaps hundreds, that participate in catecholamine synthesis, storage, release, reuptake, and metabolism, and there are perhaps thousands of SNPs that could influence sympathetic neuroeffector function. The San Diego group has reported in Circulation that there are 49 SNPs of the tyrosine hydroxylase gene alone; that a SNP for the catecholamine release–inhibiting protein catetatin is related to both altered autonomic activity and risk of hypertension; and that a common SNP of C-reactive protein may be related to blood pressure and metabolic syndrome via polymorphisms at 3 loci in catecholamine biosynthetic and adrenoceptor pathways. “SNP chip” arrays have been developed involving >1000 polymorphisms for >100 genes related to catecholamine system functions. The same investigative group has published results based on ~9800 genotypes (43 genetic variants at 17 loci) within catecholaminergic pathways. In the study of Fung et al, with only 17 polymorphisms under consideration, if the SNP search were a fishing trip, the net seemed small.

A key challenge for medical science lies in understanding how genetic changes already present at birth interact with individual life experiences and time to lead to chronic, multisystem disorders decades later, at the other side of life. Given the minute-to-minute role of the sympathetic nervous system in blood pressure responses to acute stress, it is reasonable to explore whether genetically determined alterations in catecholamine system functions contribute to chronic cardiovascular disorders such as hypertension. It is hoped that the study of Fung et al will spur further research on genotype-phenotype linkages based on catecholamine systems.

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**Disclosures**

None.

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