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PERSPECTIVE

The Confluence of Population Genetics with Molecular Pharmacology at the Angiotensin II Receptor: Dawn of a New Era or Just a New Wrinkle?

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PubMed identifies 85,590 citations for the combined keyword set of *angiotensin*, *angiotensinogen*, *renin*, and *angiotensin-converting enzyme*, yet much remains to be elucidated about the mammalian angiotensin system(s) despite nearly 100 years of research (Bader et al., 2001). This reflects several facets of this small peptide autocoid. First, angiotensin II has been linked with a diverse set of functions essential for normal mammalian physiology and apparently contributes to a variety of clinical disorders. Second, multiple tissue angiotensin systems exist, each operating through different downstream mechanisms yet often resulting in the same end physiological effects. Third, this area of research has led to multiple billion-dollar pharmaceuticals, and several of the original patents have or will soon expire, leading to efforts to find new proprietary materials. Fourth, it's a fun system to work with, because getting answers to experimental questions is not very difficult but interpreting and extrapolating the results becomes the main challenge. Last, but not least, genomic studies indicate that much of the interindividual variation in expression or functioning of this system, as well as response to system-directed pharmaceuticals, may reside in or be dictated by genetic variants (e.g., polymorphisms) (Baudin, 2002). It is this last facet that holds the promise for the future of angiotensin research and probably for all of pharmacology.

Efforts to link the angiotensin system to human disorders, primarily hypertension, has been ongoing since the discovery of renin and angiotensin. We know of two human angiotensin II receptor subtypes, AT_1 and AT_2, encoded by different genes, whereas rodents have two variants of the AT_1 subtype, AT_{1A} and AT_{1B} (Clauser et al., 1996). The majority of currently known actions of angiotensin are attributed to the AT_1 receptor, whereas functions of the AT_2, apart from its actions in the central nervous systems and during development, remain somewhat controversial (Rosenstiel et al., 2002; Wagenaar et al., 2002). With the sequencing of angiotensin, pharmacologists and biochemists began to probe structure-function relationships of what is now known as the AT_1 subtype by synthesizing thousands of sequence variants of angiotensin II and measuring affinity, efficacy, and peptide conformation. Such studies led to the development of specific antagonists and identification of critical amino acid side chains necessary for potency and efficacy. Indirectly, they also provided the first views of receptor topography. These studies were soon complemented by measurements of AT_1 signal transduction pathways and all together established the AT_1 (and AT_2) as a classic G protein-coupled receptor. Cloning of the receptors opened the door to mutagenesis studies, and soon receptor amino acids were being attributed with receptor functions—ligand binding, internalization, signal transduction, etc. What was somewhat dismaying to investigators interested in function-disorder relationships was the finding that the AT_1 receptor coupled to many alternative signaling pathways (Yin et al., 2003), leading some investigators to question why everyone was not hypertensive!

Molecular genetics opened the door for investigators to start analyzing gene sequences in search of polymorphisms, and the angiotensin system was one of the first to be probed. Single nucleotide polymorphisms (SNPs) were identified in angiotensinogen, angiotensin-converting enzyme, and the AT_1 receptor genes and population geneticists undertook association studies to test relationships of these SNPs to different cardiovascular disorders, including hypertension (Hopkins and Hunt, 2003). The initial results were chaotic; inconsistency in replication became the initial resounding message (Jeunemaitre and Gimenez-Roqueplo, 2002; Koopmans et al., 2003; Zhu and Cooper, 2003). This reflected in part the contributions of genetic

ABBREVIATIONS: SNP, single nucleotide polymorphism; cSNP, coding single nucleotide polymorphism; TM, transmembrane.

ethnicity and in part the fact that SNPs may be silent or neutral and without overt influence on gene product or gene-function relationships. That mutations or genetic polymorphisms may not translate into altered gene functioning has been appreciated by geneticists for years, yet such conclusions were less familiar to bench molecular biologists who could point to studies in which single nucleotide mutagenesis resulted in significantly altered receptor functioning in vitro and even in vivo. Thus, many published single base changes are mainly of academic interest and should not be extrapolated as potential disease contributors. What has been lacking for the pharmacologist was the frequency of occurrence of SNPs, around 8 ± 2 per 10 kilobases for humans (Zhao et al., 2003a), and knowledge of the gene polymorphisms that existed in nature (in humans or model organisms). The shift of the human and model organism genome projects to construction of databases of SNPs, construction of SNP maps, and recently to construction of haplotype blocks has changed the scene for all pharmacologists. Genomics and molecular and population genetics, which have been circling the "field of engagement" for the past 10 years, have arrived at center stage of molecular pharmacology—and our world will never be the same!

The current issue contains a report from Hansen et al. (2004) detailing studies of a series of AT_1 receptor variants engineered to replicate reported coding polymorphisms (cSNPs) identified in a series of different population genetic studies. These cSNPs were examined originally for association with different human (cardiovascular) disorders ranging from genetic hypertension, diabetic nephropathy, renal dysfunction, coronary constriction, and left ventricular hypertrophy to cerebrovascular disease (see article for references). The experimental logic behind the current study was beautifully simple. First, an assumption was made that nonsynonymous polymorphisms (i.e., those that result in coding changes) would more probably be associated with altered gene product functions and diseases. This was based on an earlier study by the same group (Lee et al., 2003). Second, given that these cSNPs of the AT_1 receptor have been established within subsets of human populations with disease (albeit at a minor allelic frequency), if one created the equivalent receptor gene variant, expressed it in a defined in vitro cell system and tested it for altered receptor properties—ligand affinity, efficacy, and signal transduction—one could discern the relative importance of each polymorphism to receptor function. Thus, results of epidemiological association studies were converted into classic hypothesis-driven bench research. Of course, this is not the first time that a human SNP was replicated in an in vitro model for study. The difference here is that the study design was to compare and contrast a *set* of cSNPs all from a single gene.

Seven receptor gene variants were synthesized and tested, first in a high-throughput cellular screening assay (termed receptor selection and amplification technology), then by direct cellular ligand binding, and finally for altered signaling focusing on inositol phosphate and extracellular signal-regulated kinase phosphorylation assays as downstream readouts. Three of the cSNPs were within transmembrane (TM) spanning domains of the receptor, one each in TM1, TM5, and TM6, whereas four were in the intracellular carboxyl terminus (Fig. 1). The receptor selection and amplification technology assay identified the three TM polymorphisms as different from wild type, whereas the four carboxyl terminal polymorphisms seemed neutral. Of the three TM coding changes, none interfered with cell surface expression, whereas one, G45R in TM1, completely abolished receptor affinity and efficacy. A cysteine-to-tryptophan mutation (C289W) reduced potency but not efficacy, whereas a phenylalanine-to-serine mutation (F204S) reduced both potency and efficacy. Thus, all three coding changes could be classified as leading to "loss of function". The glycine-to-arginine polymorphism at position 45 was first reported by Rolfs et al. (1994) from a study of human control subjects, with no evident cardiovascular disease in the test subjects or first-degree relatives. This site is localized in the TM1 domain near the first intracellular loop. Given that the change is from a polar amino acid with minimal volume demands to a charged, extended side chain amino acid, it is perhaps not surprising that it disrupts key receptor functions, such as ligand binding and transduction. Although the authors speculate that the G45R polymorphism may be determined (in the future) to be a "disease-related" (polymorphism) for AT_1 receptor physiology, it is unclear why the "control" subjects who carried this polymorphism exhibit no evident pathology. In rodents, the AT_{1B} can subserve many of the functions of the AT_{1A} in mice devoid of AT_{1A} (Oliverio et al., 1997); however, AT_{1A}-null mice exhibit hypertrophy of the juxtaglomerular apparatus and glomerular mesangial expansion along with evidence of a "survival disadvantage" (Oliverio et al., 1998). As far as is known, loss of the AT_1 in humans would not be compensated for by an alternate AT_1 variant.

Hansen et al. (2004) raise more questions than answers, as good research should. Would single polymorphisms of membrane receptors be expected to exert incrementally measurable effects on receptor function? Data from the current article could argue in either direction. The four coding changes in the carboxyl terminus did not alter measured receptor properties, implying that a single cSNP may indeed be neutral and not additive: however, what if other receptor functions were altered, but were missed in this study because they were not measured? Thus, what is the most appropriate battery of in vitro tests for subtly altered receptor functions? The C289W polymorphism reduced only potency, which agrees with studies showing that this segment of the receptor polypeptide interacts with peptide agonist (Perodin et al., 2002), whereas the F204S polymorphism reduced both potency and efficacy. Do these reductions translate into meaningful alterations in organ system or whole organism functions? What evidence would be sufficient to warrant creation of engineered model organisms, and should it be one coding change per strain? More importantly for angiotensin researchers is how "loss of function" translates into altered angiotensin system function? It is correct that changes in potency for the agonist could imply altered pharmacogenomic potential, but disorders associated with the angiotensin system (e.g., genetic hypertension) are generally attributed to abnormal "gain of function." As shown in AT_{1A} receptor

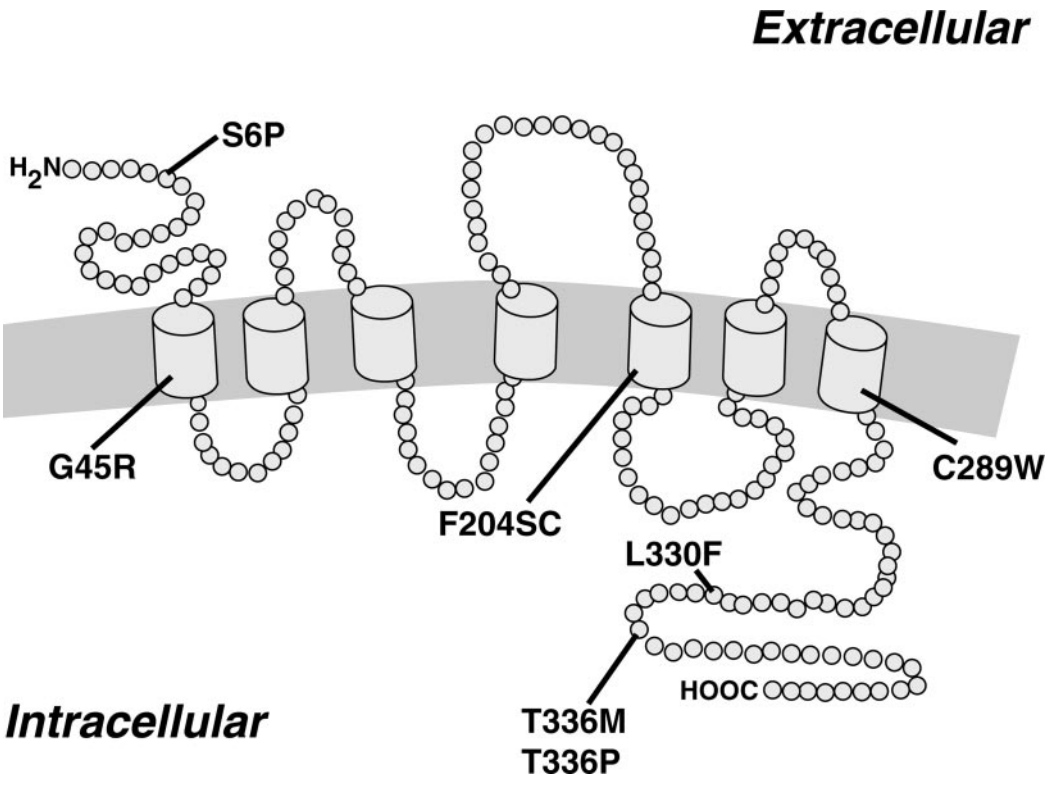

Fig. 1. Schematic of AT_1 illustrating location of seven polymorphisms tested. [Adapted from Kai H, Alexander RW, Ushio-Fukai M, Lyons PR, Akers M, and Griendling KK (1998) G-protein binding domains of the angiotensin II AT_{1A} receptors mapped with synthetic peptides selected from the receptor sequence. *Biochem J* **332:**781–787.]

knockout studies, "loss of function" could be beneficial in some disorders, not detrimental (Cervenka et al., 2002). Thus, could such coding changes offer an "advantage" to organisms, rather than result in disease?

Pharmacological researchers, whether molecular, physiological or genomic, must now design studies that incorporate knowledge learned from genetic studies and analyses of human and model organisms (including but not limited to rat and mouse). For traits and diseases dictated by complex genetics, individual SNPs are likely to have little impact from a population genetic standpoint (Zhao et al., 2003b). Rather, haplotypes, and perhaps even combinations of haplotypes in different genes, are probably needed to answer the ultimate question of what underlies real biological variability (Gabriel et al., 2002). If this is the case, future studies, like the current one, should focus on in vitro haplotype constructs so as to better replicate potential disease processes or complex traits. Finally, Hansen et al. (2004) illustrate that in the broad picture of pharmacological research, the role and importance of genetic background in experimental design must begin to be taken into consideration. Genetic background effects can introduce significant confounds if the question being asked extends to the whole cell, organ system, or beyond (Le Corvoisier et al., 2003; Monti et al., 2003; Printz et al., 2003). These and other lessons gleaned from Hansen et al. (2004) argue the dawn of a new era, and a very challenging one at that.

References

Bader M, Peters J, Baltatu O, Muller DN, Luft FC, and Ganten D (2001) Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med* **79:**76–102.

Baudin B (2002) Angiotensin II receptor polymorphisms in hypertension. Pharmacogenomic considerations. *Pharmacogenomics* **3:**65–73.

Cervenka L, Horacek V, Vaneckova L, Hubacek JA, Oliverio MI, Coffman TM, and Navar LG (2002) Essential role of AT_{1A} receptor in the development of 2K1C hypertension. *Hypertension* **40:**735–741.

Clauser E, Curnow KM, Davies E, Conchon S, Teutsch B, Vianello B, Monnot C, and Corvol P (1996) Angiotensin II receptors: protein and gene structures, expression and potential pathological involvements. *Eur J Endocrinol* **134:** 403–411.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. (2002) The structure of haplotype blocks in the human genome. *Science (Wash DC)* **296:**2225–2229.

Hopkins PN and Hunt SC (2003) Genetics of hypertension. *Genet Med* **5:**413–429.

Jeunemaitre X and Gimenez-Roqueplo AP (2002) Genetics and arterial hypertension: 3 approaches to decode a complex disease. *Bull Acad Natl Med* **186:**1595–1606.

Kai H, Alexander RW, Ushio-Fukai M, Lyons PR, Akers M, and Griendling KK (1998) G-Protein binding domains of the Angiotensin II AT_{1A} receptors mapped with synthetic peptides selected from the receptor sequence. *Biochem J* **332:**781–787.

Koopmans RP, Insel PA, and Michel MC (2003) Pharmacogenetics of hypertension treatment: a structured review. *Pharmacogenetics* **13:**705–713.

Le Corvoisier P, Park HY, Carlson KM, Marchuk DA, and Rockman HA (2003) Multiple quantitative trait loci modify the heart failure phenotype in murine cardiomyopathy. *Hum Mol Genet* **12:**3097–4107.

Lee A, Rana BK, Schiffer HH, Schork NJ, Brann MR, Insel PA, and Weiner DM (2003) Distribution analysis of nonsynonymous polymorphisms within the G-protein-coupled receptor gene family. *Genomics* **81:**245–248.

Monti J, Plehm R, Schulz H, Ganten D, Kreutz R, and Hubner N (2003) Interaction between blood pressure quantitative trait loci in rats in which trait variation at chromosome 1 is conditional upon a specific allele at chromosome 10. *Hum Mol Genet* **12:**435–439.

Oliverio MI, Best CF, Kim HS, Arendshorst WJ, Smithies O, and Coffman TM (1997) Angiotensin II responses in AT_{1A} receptor-deficient mice: a role for AT_{1B} receptors in blood pressure regulation. *Am J Physiol* **272:**F515–F520.

Oliverio MI, Madsen K, Best CF, Ito M, Maeda N, Smithies O, and Coffman TM (1998) Renal growth and development in mice lacking AT_{1A} receptors for angiotensin II. *Am J Physiol* **274:**F43–F50.

Perodin J, Deraet M, Auger-Messier M, Boucard AA, Rihakova L, Beaulieu M-E, Lavigne P, Parent J-L, Guillemette G, Leduc R, et al. (2002) Residues 293 and 294 are ligand contact points of the human angiotensin type 1 Receptor. *Biochemistry* **41:**14348–14356.

Printz MP, Jirout M, Jaworski R, Alemayehu A, and Kren V (2003) Genetic models in applied physiology. HXB/BXH rat recombinant inbred strain platform: a newly enhanced tool for cardiovascular, behavioral and developmental genetics and genomics. *J Appl Physiol* **94:**2510–2522.

Rolfs A, Weber-Rolfs IW, Regitz-Zagrosek V, Kallisch H, Riedel K, and Fleck E (1994) Genetic polymorphisms of the angiotensin II type A (AT_1) receptor gene. *Eur Heart J* **15:**(Suppl D)**:**108–112.

Rosenstiel P, Gallinat S, Arlt A, Unger T, Sievers J, and Lucius R (2002) Angiotensin AT2 receptor ligands: do they have potential as future treatments for neurological disease? *CNS Drugs* **16:**145–153.
Wagenaar LJ, Voors AA, Buikema H, and van Gilst WH (2002) Angiotensin receptors in the cardiovascular system. *Can J Cardiol* **18:**1331–1339.
Yin G, Yan C, and Berk BC (2003) Angiotensin II signaling pathways mediated by tyrosine kinases. *Int J Biochem Cell Biol* **35:**780–783.
Zhao Z, Fu YX, Hewett-Emmett D, and Boerwinkle E (2003a) Investigating single nucleotide polymorphism (SNP) density in the human genome and its implications for molecular evolution. *Gene* **312:**207–213.
Zhao LP, Li SS, and Khalid N (2003b) A method for the assessment of disease associations with single-nucleotide polymorphism haplotypes and environmental variables in case-control studies. *Am J Hum Genet* **72:**1231–1250.
Zhu X and Cooper RS (2003) Linkage disequilibrium analysis of the renin-angiotensin system genes. *Curr Hypertens Rep* **5:**40–46.

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