Implications of Species Difference for Clinical Investigation
Studies on the Renin-Angiotensin System

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Abstract—The justification for clinical investigation has its roots in the fact that physiological mechanisms and disease pathogenesis in animal models replicate mechanisms and pathogenesis in humans only in part. In the case of the renin-angiotensin system, there is species variation in the anatomic distribution of the renin-angiotensin system, in the active site of the renin enzyme, and in the structure of angiotensin and the AT\(_1\) receptor. The conversion of angiotensin I (Ang I) to angiotensin II (Ang II) may prove to be the most important aspect of species variation. In plasma, all the conversion occurs through a single enzyme, angiotensin-converting enzyme (ACE), and species variation in structure and function have not been reported. Non–ACE-dependent pathways, which occur only at the tissue level, show unambiguous, striking species variation. Specifically, chymase, the most important enzyme responsible for non–ACE conversion of Ang I to Ang II, shows striking species variation. In humans and a number of species, including the hamster, quantitatively important chymase-independent Ang II formation from Ang I occurs in the heart, arteries, and kidney. In rats and rabbits, on the other hand, chymase differs, is not active in the conversion of Ang I to Ang II, and indeed is involved in Ang II degradation. Consequently, one would anticipate that blockade of the system at the ACE step would be equivalent to that at the Ang II receptor in the rat. This has been widely reported. In humans, on the other hand, one would anticipate that the AT\(_1\) receptor blockers will be more effective than ACE inhibitors. Again, preliminary evidence favors this possibility. The implications for therapeutics are clear. (Hypertension. 2000;35[part 2]:150-154.)

Key Words: angiotensin I ■ angiotensin II ■ angiotensin-converting enzyme ■ human ■ rats ■ rabbits ■ hamsters

Animal models—studies in rats, mice, and other small creatures—have made an enormous contribution to our understanding of normal physiology and to disease expression. If findings in rats and mice always predicted what we would find in humans, for most of us there would be little justification for studies in humans. Conversely, if rats and mice never predicted what we would find in humans, there would be little rationale for studying mechanisms in rats and mice. An important responsibility of clinical investigation, some might say the major responsibility, involves identifying when studies in animal models have led us astray and when they have predicted human mechanisms.

The renin-angiotensin-aldosterone system provides a large series of examples of variation with species,\(^1-4\) some of which may well have crucial clinical implications, the thrust of this article.

Sokabe\(^4\) in 1974 summarized what was known of the phylogeny of the renin system. Juxtaglomerular cells were demonstrated early in phylogeny, in holocephali, when fish first learned to create bone, and thus the nervous system was protected. Thereafter, in various saltwater and freshwater fish, amphibians, reptiles, and birds, the anatomic position of the juxtaglomerular cells was always in the afferent elements of the arterial blood supply. An extension to the efferent arteriole emerged only in mammals.\(^4\) Presumably, with that anatomic shift, an accompanying shift in the functional role of the renin system in the control of glomerular capillary perfusion and hydrostatic pressure followed.

Renin, angiotensin I (Ang I), and angiotensin II (Ang II) have been recognized to show differences in structure between species for >30 years.\(^1-3\) In the case of renin, the structural variations are more substantial and make for striking species specificity. As an example, the development of renin inhibitors was influenced strongly by species, because the target had to be primate renin.\(^5,6\) Consequently, all the supporting laboratory studies had to be performed in primates, with a need to develop new models and at substantial expense. Renin inhibitors have been developed for the rat, primarily because so many of our useful models have been developed in the rat.\(^7\)

In the case of Ang I and Ang II, the species variation in structure appears to have had no functional implications. Two natural angiotensin molecules have been identified, differing only in the fifth amino acid: Ile\(^5\) and Val.\(^5\) Human, hog, and rat Ang II contains Ile\(^5\); ox Ang II contains Val.\(^5\) The latter has been the most widely applied because of availability. The chemical structures of angiotensin-like agents in amphibians, birds, reptiles, and teleosts appear to differ more substantially than do mammalian angiotensins.\(^8\)
The structure and function of the angiotensin receptor in different species is still emerging, but the available evidence concerning AT\textsubscript{1} receptors and their response to AT\textsubscript{1} receptor antagonists suggests a very similar series of mechanisms in small animals and in humans.\textsuperscript{3,9} The AT\textsubscript{1} receptor was first cloned in vascular smooth muscle cells\textsuperscript{10} and beef adrenal gland.\textsuperscript{11} In the rat, the AT\textsubscript{1} receptor has been shown to possess 2 isoforms, AT\textsubscript{1A} and AT\textsubscript{1B}.\textsuperscript{12} The 2 isoforms share a high degree of homology differing mainly in the noncoding portion of the gene and are located on 2 different chromosomes. In humans, on the other hand, a single gene codes for the AT\textsubscript{1} receptor.\textsuperscript{13} The AT\textsubscript{1} receptor also shows marked conservation between species in the mouse, rat, and human.\textsuperscript{14} In view of the striking similarity of the influence of AT\textsubscript{1} antagonists in different species, including humans, it is unlikely that quantitatively important differences will be found between species.\textsuperscript{3,9,15}

It is in the conversion of Ang I to Ang II that the most striking and important species differences emerge. The differences appear to express themselves in 2 ways. First, there is reasonable evidence to suggest that the mechanism through which angiotensin-converting enzyme (ACE) inhibitors influence the renal blood supply differs by species. In some species, the involvement of bradykinin accumulation appears to be substantial, whereas in other species, it appears to be a minor theme. Perhaps more important, there is evidence that non–ACE-dependent pathways for Ang II generation also differ by species. These findings have important implications for therapeutics.

### Species Differences in the Contribution of Kinins, Prostaglandins, and Nitric Oxide to the Renal Vascular Response to ACE Inhibition

A series of impressive but mixed observations suggests a substantial contribution of kinins to the renal vascular response to ACE inhibition in at least some animal models. A reasonable interpretation of the differences in these studies is that there is quantitatively important species variation. ACE inhibition, for example, increased prostaglandin production in the canine\textsuperscript{16} but not rabbit\textsuperscript{17} kidney. This finding would suggest that the renal vasodilatation that followed ACE inhibition involved different mechanisms in these species. Is that hypothesis testable? Is the renal vasodilator response to ACE inhibition in the rabbit more dependent on reduction in Ang II formation than in the other species? If so, one would anticipate that an Ang II antagonist would blunt renal vasodilator responses to ACE inhibitors more in the dog than in the rabbit. Indeed, in similar protocols, a partial agonist Ang II antagonist blunted the renal blood flow response to ACE inhibition in the dog and rat\textsuperscript{18–20} but not in the rabbit.\textsuperscript{21} As an alternative test, one would anticipate that bradykinin antagonists would have a larger influence on the renal blood flow response to ACE inhibition in some species than in others. This is precisely what was found in dogs and rats\textsuperscript{16,22} but once again not in the rabbit\textsuperscript{23,24} in the degree to which bradykinin antagonists blunted the renal hemodynamic response to ACE inhibition. In the rat, it is primarily medullary perfusion that is kinin dependent.\textsuperscript{25} Thus, apparent species differences may reflect the relative contribution of medullary perfusion to total renal blood flow. In this case, humans resemble rabbits far more than they do the rat or dog.\textsuperscript{26,27} Whatever the explanation, it is clear that one cannot extrapolate from studies on mechanisms by which the kidney responds to ACE inhibitors in animal models to the control of renal circulation in humans—even in health and much less when disease is superimposed.

### Species Differences in Local Ang II-Forming Pathways

The vast majority of studies comparing the effectiveness of ACE inhibitors and AT\textsubscript{1} receptor antagonists have been made in rat models of cardiovascular disease. In the rat, these 2 classes of inhibitor generally lower blood pressure and prevent cardiovascular remodeling to a similar degree. There are, however, important species differences in local Ang II-forming pathways in the cardiovascular system, mainly because of different characteristics of chymases.\textsuperscript{28–32} Human and hamster chymases hydrolyze Ang I to form Ang II, whereas rat chymase not only does not hydrolyze Ang I to Ang II but also participates in the degradation of Ang II.\textsuperscript{33} Thus, one would anticipate that ACE inhibitors and Ang II antagonists would induce an identical physiological response in the rat. Virtually all the Ang II generation in the rat occurs via the ACE pathway.

In accord with these in vitro studies on chemical pathways, the physiological evidence for alternative pathways first emerged from studies in the hamster.\textsuperscript{34} Cornish et al\textsuperscript{35} found that vasoconstriction induced by Ang I in the blood vessels of hamster cheek pouch was inhibited only partially by ACE inhibitors in high concentration but was completely inhibited by either an Ang II receptor antagonist or an antiserum directed against Ang II. The character of the enzyme or enzymes responsible for conversion of Ang I to Ang II remained unclear.

Between 1984 and 1990, Okunishi et al\textsuperscript{35,36} and Okamura and coworkers\textsuperscript{37} described evidence from studies of blood vessels of humans, monkeys, and dogs for a unique enzyme that converts Ang I to Ang II but differs from ACE.\textsuperscript{35–37} Their observation that this conversion was catalyzed by an enzyme that was inhibited by several serine-protease inhibitors, including chymostatin, provided a clue as to the nature of the enzyme. In their studies, chymostatin in high concentration provided partial blockade of the conversion of Ang I to Ang II. Captopril or other ACE inhibitors also provided partial inhibition, although somewhat less than that induced by chymostatin, and the combination of chymostatin and ACE inhibition led to total blockade of Ang II formation in primate and canine blood vessels.\textsuperscript{28} Their primary experimental end point was the contractile response of isolated blood vessels to Ang I in vitro.

These researchers\textsuperscript{35–37} designated the newly found enzyme responsible for converting Ang I to Ang II as CAGE, an acronym obtained from the description chymostatin-sensitive Ang II-generating enzyme. Evidence was assembled that this enzyme represented a chymase derived from passenger mast cells located in the adventitia of the arterial segments studied in vitro, presumably a cellular passenger.\textsuperscript{38} These unambig-
uous facts led to an area of investigative concern. Was it likely that an enzyme derived from mast cells plays a role in normal physiology? As a second concern in the in vitro experiment in which Ang I is injected into the tissue bath surrounding the artery, the resultant hormone concentrations in the adventitia at the antiluminal surface are as high as they are in the lumen near the media where the contractile apparatus operates. In vivo, if Ang I is generated primarily in the circulation rather than locally, the Ang I concentration in the adventitial interstitium might be too low for CAGE to make an important functional contribution.

Even more fundamentally, in a series of reports over that same time interval, other investigators were unable to confirm the findings of Okunishi et al. Each study failed to demonstrate any evidence for the presence of non-ACE enzymatic pathways in the vasculature, because the responses to Ang I were completely abolished by ACE inhibition. In view of the simplicity and wide use of the preparations, it seemed unlikely that technical factors were responsible.

In a crucial follow-up report, Okunishi et al. accounted for the differences in an elegant study that has raised crucial issues for future investigators. They noted that the studies that failed to confirm their original observations had been performed with rat or rabbit blood vessels. Their follow-up study, which was designed to address the issues raised by that difference in study design, is well described in the title of their report, “Marked Species-Difference in the Vascular Ang II-Forming Pathways: Humans Versus Rodents.” In isolated arteries, they demonstrated a marked difference in the pathways for Ang II formation between human, rat, and rabbit arteries. In human gastroepiploic arterial strips, treatment with captopril blocked only 30% to 40% of the conversion of Ang I to Ang II. Treatment with chymostatin blocked about 60% of Ang II generation. A combination of captopril and chymostatin was required to produce 100% blockade. In rabbit arteries, on the other hand, captopril induced >90% inhibition, and chymostatin had little or no effect. One technical concern was that the smaller arteries from the rabbit would suffer more endothelial damage or loss, but Okunishi et al. provided both morphological and functional evidence for the integrity of endothelium in all their preparations. They made the interesting speculation that their observation might account for the disturbing inability of ACE inhibitors to prevent the arterial response to injury in primates, despite the interesting speculation that their observation might make the interesting speculation that their observation might make an important functional contribution.

Our initial anticipated result was that the renal hemodynamic response to ACE inhibition under these circumstances reflected not only the decrease in local Ang II formation but also reduced kinin degradation. The result would be the accumulation of vasodilator products, including bradykinin and kinin-dependent prostaglandin formation, or activation of endothelial nitric oxide release. To our surprise, the renal vasodilator response to the renin inhibitor enalapril was remarkable, exceeding expectations from our experience with ACE inhibitors. In a follow-up, 3-arm study that in random order compared double-blind responses to placebo, captopril, and the same renin inhibitor, enalapril, placebo did nothing, and captopril and enalapril both led to renal vasodilation. The response to enalapril was larger than the response to captopril in 6 of 9 healthy subjects, confirming our earlier observation. These findings with enalapril in 2 studies were supported by a third study that used zamipril as the renin inhibitor in the same model.

Although renin is a fastidious enzyme with great substrate specificity, a possible interpretation of our findings was that the renin inhibitors acted by an action unrelated to renin. Several lines of investigation make this unlikely. Ang II administration into the renal arteries in dogs after renin inhibition completely reversed the diuresis and natriuresis induced by the renin inhibitor. In accord is the observation in humans of blunting of the renal vascular response to renin inhibition by a high salt diet and in low renin hypertension and concordance in the primary renal vasodilator response to ACE and renin inhibition. Despite all these considerations, the possibility existed that renin inhibition led to an overestimation of the contribution of the renin-angiotensin system to renal vascular tone because of a lack of specificity, reflecting an action unrelated to renin.

In this context, the development of the Ang II antagonist class created the possibility of a “tiebreaker.” If the renin inhibitor acted via an alternative non-angiotensin-dependent mechanism, one would anticipate that Ang II antagonists would provide a different renal vascular response under the
conditions of our study. Conversely, if the renin inhibitor acted only through blockade of renin-dependent Ang II formation, one would anticipate an identical response to the renin inhibitor and Ang II antagonist. We have studied 3 Ang II antagonists in that model, eprosartan, Irbesartan, and candesartan, and in each case have defined the relation between Ang II antagonist dose and response. At the top of the dose-response relationship, both Ang II antagonists induced a response that agreed with the response to the renin inhibitor.50,51

The most parsimonious interpretation of our finding—that multiple renin inhibitors and Ang II antagonists induce an almost-identical renal vascular response in humans that substantially exceeds the response to ACE inhibition—suggests that a renin-dependent but ACE-independent pathway for Ang II generation is involved. From the blood flow ratios, one can calculate that about two thirds of Ang II formation in the healthy human kidneys in which Ang II formation has been stimulated by a low salt diet occurs via the ACE pathway, and about one third occurs via non–ACE-dependent pathways. Thus, the non–ACE-dependent pathway would be less than that in intact isolated human arteries.28 At the moment, in the light of the studies reviewed in this article, it is reasonable to attribute those responses to chymase or to CACE, a chymase-like enzyme.

Perhaps most important, these observations have implications for therapeutics. If Ang II is a toxin under some circumstances, the possibility that blocking the system by renin inhibition or Ang II antagonism will provide greater efficacy than does ACE inhibition requires exploration. Our studies in diabetes, moreover, raise the interesting possibility that these non–ACE-dependent pathways become quantitatively more important under conditions of disease.50 That would place an even higher priority on the therapeutic trials with alternative blockers.

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