

## Review

# Pathophysiologic Implications of Angiotensin I-Converting Enzyme as a Mechanosensor: Diabetes

D.W. MOSKOWITZ, M.D.

### ABSTRACT

If somatic angiotensin I-converting enzyme (ACE) were a mechanosensor, as recently claimed, it would provide insight into the molecular origin of most adult diseases, such as diabetes, cancer, autoimmune diseases, and psychiatric disease, as well as aging itself. The "ACE as mechanosensor" hypothesis holds that tissue ACE is activated by turbulent flow with each heart beat, so that age-dependent diseases begin with a signal from the vasculature. Activation of ACE would thus represent the first of many amplification steps ("cascades"), placing it at the origin of most age-dependent diseases. As a corollary, effective inhibition of tissue ACE might significantly delay the progression of most diseases of aging. In this paper we will explore how useful this hypothesis is in explaining the molecular pathogenesis of diabetes and its complications, in which aging is accelerated.

### NON-INSULIN-DEPENDENT DIABETES MELLITUS

**T**HE WORLDWIDE INCIDENCE of type 2 diabetes [non-insulin-dependent diabetes mellitus (NIDDM)] is increasing. In communities like the Pima Indians, NIDDM was rare two generations ago, but now has a prevalence of 50%.<sup>1,2</sup> NIDDM thus appears to be the result of modern Western living (a high-calorie diet and relative physical inactivity) superimposed on a "thrifty" genotype.<sup>3</sup> We shall see how angiotensin I-converting enzyme (ACE) as a mechanosensor helps explain this epidemiologic observation.

#### *Survival advantage of insulin resistance*

During most of the past 7 million years of our species' existence, humans existed in a state

of relative starvation. Hunting and fishing techniques were extremely inefficient. So was agriculture, which was discovered only in the last 15,000 years. Amerindians who came to the Americas from Asia beginning 30,000 years ago had to have a "thrifty genotype" to survive. Insulin resistance is a key component of the thrifty genotype.<sup>3</sup>

Insulin is a hormone designed for caloric plenty, a "fiesta" hormone. During starvation, triglycerides are broken down to free fatty acids. This occurs every time we go to sleep, or fast for longer than about 6 h. When fasting lasts more than a couple of weeks, free fatty acids become the fuel for all organs of the body, including the brain. Only red blood cells continue to use glucose as their major fuel during prolonged starvation.

In its antilipolytic role, insulin promotes stor-

age of dietary fats as triglycerides. Insulin promotes the uptake of dietary glucose by the muscle and liver, and its storage as the relatively short-term energy source, glycogen. Insulin thus only helps the organism if glucose and fat are in the diet. Early humans ate a diet high in fiber (e.g., roots and tubers), but quite low in calories. In the absence of dietary glucose, insulin activity promotes hypoglycemia, activation of the sympathoadrenal axis, massive increase<sup>4</sup> in plasma epinephrine (which goes up 60-fold) and norepinephrine (which increases threefold), and the attendant symptoms of anxiety and tachycardia (i.e., the “fight or flight” response).

To survive during millions of years of starvation, the species had to be resistant to insulin. Insulin resistance had to be the norm, not the exception. The “party hormone” had to be turned off, since for over 99% of our existence as a species, life was no party. Indeed, caloric plenty did not become available in the United States until the beginning of the 20th century. The Pima Indians saw caloric plenty only within the past two generations.<sup>1-3</sup> The party of caloric plenty is just beginning in the Third World, suggesting that the epidemic of NIDDM will soon be global.

Our species, like all other animals, was also hard-wired to detect calorically rich foods. Hence our insatiable appetite for processed sugar, as well as high-cholesterol, fatty foods like red meat. Our taste buds and olfactory system, and the organization of our brain, reflect these priorities. Every medical student is familiar with the “homunculus” of sensory innervation represented in our cerebral cortex: One-third of the homunculus is the mouth.

#### *The molecular mechanism of insulin resistance*

For at least half a billion years,<sup>5</sup> angiotensin II appears to have been the organism’s primary mechanism for ignoring insulin. Angiotensin II, through activation of protein kinase C (PKC), decreases signaling by the insulin receptor due to serine/threonine phosphorylation of the insulin receptor itself and its downstream substrates.<sup>6</sup>

The rate-limiting step in angiotensin II production appears to be activity of ACE.<sup>7</sup> Nor-

mally, the N-terminal active site of ACE is hypothesized to bind an autoinhibitory oligopeptide, and must be activated by turbulent flow.<sup>5</sup> How is the N-terminal active site of ACE activated by diet?

Ingestion of sugars, fats, or salt (another environmentally scarce commodity except among the Pacific Islanders; cf. Roman “salary”) raises the osmolality ( $\mu$ ) of the blood. Most of plasma osmolality is determined by the plasma concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and albumin, which are rather tightly controlled by the organism. The effect on plasma osmolality of dietary sugars, fats, and salt is expected to be minor.<sup>5</sup>

But in the absence of available fresh water, which was historically the case for all human settlements except along rivers, plasma  $[\text{Na}^+]$  and  $[\text{Cl}^-]$  will vary considerably during the day, especially after high-salt meals. Clinically, it is common to see elderly patients, whose thirst is known to be decreased,<sup>8</sup> placed on diuretics, and who have no symptoms of increased thirst despite a serum osmolality of 310 mOsm/kg of water. It is also not unusual to see volume-depleted elderly patients receiving diuretics and insufficient dietary salt whose  $[\text{Na}^+]$  is 110 mEq/L instead of 140 mEq/L, but who have no symptoms, the so-called “syndrome of appropriate antidiuretic hormone.”

These commonly encountered clinical situations were probably also common among prehistoric humans without ready access to water. It is likely that prehistoric humans had wide excursions in their serum osmolality, perhaps over the range of 230–320 mOsm/kg of water. Only in people with access to free water is the osmolality maintained narrowly at  $283 \pm 3$  mOsm/kg of water, as advertised in medical textbooks.

The effect of ingesting osmotically active nutrients like sugars, fats, or salt may be small per se, since doubling serum glucose concentration from 100 mg/dL to 200 mg/dL causes only a 1% increase in osmolality.<sup>5</sup> But they induce an obligate osmotic diuresis, depleting the organism of its free water. The effect can only be reversed by drinking free water, which historically was unavailable unless one lived a few feet from a lake or river. The critical role of plasma osmolality in circulatory hemodynamics has long been recognized.<sup>9</sup>

Thus, a 10% increase in plasma osmolality from 283 to 310 mOsm/kg of water would not be at all unexpected after a calorically rich meal. Since shear stress  $\tau$  is directly proportional to osmolality  $\mu$ ,<sup>5</sup> a 10% increase in the latter should result in a similar increase in the former.

Shear stress (blood velocity) in areas of turbulent flow is hypothesized to provide the mechanical force that activates the N-terminal active site of ACE.<sup>5</sup> Assuming the relationships are all linear, activation of 10% more ACE molecules would lead to a 10% increase in local angiotensin II concentration, leading to 10% more occupancy of angiotensin type 1 (and type 2) receptors.

At this point, linearity probably ceases to obtain, since we are dealing with a signaling cascade. A 10% increase in AT1 receptor occupancy may lead to more than a 10% increase in PKC activation, which in turn will lead to more than a 10% desensitization of insulin receptor signaling.<sup>6</sup>

The more ACE molecules expressed on the endothelial cell membranes of arteries, arterioles, and capillaries in skeletal muscle, and on endothelial cell membranes of the portal vein leading from the gut to the liver, the more ACE molecules will be activated by an increase in osmolality from an osmotically rich meal. For this reason, people who have a genomic polymorphism resulting in greater transcription of the ACE gene [i.e., the ACE deletion/deletion (*D/D*) genotype] are expected to have a higher degree of insulin resistance. We have observed a mildly elevated ( $\sim 1.2$ ) odds ratio (OR) for Caucasians and NIDDM.<sup>10</sup>

Angiotensin II is a well-known vasoconstrictor and anti-natriuretic factor. Insulin also is anti-natriuretic.<sup>11</sup> Angiotensin II promotes insulin resistance, which in turn promotes hyperinsulinism. The combination of elevated angiotensin II and insulin concentrations resets the pressure–natriuresis curve, resulting in systemic hypertension.<sup>12</sup> This explains why essential hypertension and insulin resistance frequently coincide in the same patient.<sup>13</sup>

The activation of endothelial ACE to produce angiotensin II, which is both pressor and cause of insulin resistance, can easily explain the frequency of hypertension among patients with

NIDDM. Elevated angiotensin II levels can also explain the accelerated atherosclerosis seen in essential hypertension, which is even more accelerated by hyperglycemia in NIDDM.<sup>5</sup> But why are there 60 million Americans with essential hypertension and insulin resistance but only 20 million Americans with overt NIDDM? What more is required to establish hyperglycemia?

#### *Pancreatic $\beta$ -cell apoptosis*

Insulin resistance, which is hypothesized to be due to activation of ACE by osmotically active meals, is known to result in hyperinsulinism. Pancreatic islets of Langerhans undergo cellular hypertrophy and hyperplasia. The number and size of insulin granules within  $\beta$ -cells increase, as does the number of insulin-containing cells. Glucose itself is mitogenic,<sup>14</sup> as is insulin.<sup>15</sup>

A clue to what happens in NIDDM comes from the rare congenital syndrome of hyperinsulinism. Hyperinsulinism is due to inappropriate pancreatic  $\beta$ -cell hypertrophy and hyperplasia (either diffuse or adenomatous). With time, there is increasing islet cell apoptosis.<sup>16</sup> What causes  $\beta$ -cell apoptosis in NIDDM?<sup>17</sup> And why don't all patients with essential hypertension and insulin resistance lose sufficient  $\beta$ -cells to become overtly hyperglycemic?

Apoptosis of pancreatic  $\beta$ -cells appears to be the price paid for hypoglycemia ("insulin overshoot"), not hyperglycemia. For this line of reasoning I am indebted to Steve Giddings, who observed very little gene activation in pancreata of glucose- and insulin-clamped hyperglycemic rats, but profound induction of apoptosis-related genes such as *hsp70* after brief episodes of hypoglycemia (personal communication).

Hypoglycemia is strongly guarded against by the organism, since acutely the organism is entirely dependent on glucose as fuel. Hypothalamic glucose-sensing neurons, operating through spinal nerves containing AT1 receptors, activate sympathetic nerves ending in the adrenal gland.<sup>4</sup> The adrenal medulla releases epinephrine, raising the plasma concentration 60-fold. Sympathetic nervous discharge raises plasma norepinephrine threefold.

The same hypothalamic glucose-sensing neurons may be responsible for release of ACTH, which acts on the adrenal cortex to release cortisol, another counterregulatory hormone, which elevates plasma glucose, in concert with plasma epinephrine and glucagon. Glucagon release occurs after sympathetic nervous stimulation of pancreatic  $\alpha$ -cells.

The huge rise in plasma catecholamines increases blood sugar, as well as heart rate and blood pressure. Animals with the highest increase in plasma norepinephrine are the ones predisposed to develop overt diabetes.<sup>18</sup>

With increased blood pressure and heart rate, turbulent blood flow increases within the systemic arterial vasculature, leading to more activation of endothelial ACE according to our hypothesis.<sup>5</sup>

The pancreas contains a tissue renin-angiotensin system, and  $\beta$ -cells express AT1 receptors.<sup>19</sup> Angiotensin II suppresses insulin release by causing vasoconstriction acutely.<sup>20</sup> In the long term, angiotensin II likely induces apoptosis of  $\beta$ -cells. Apoptosis, although usually thought of in the context of AT2 receptors, can also be mediated by AT1 receptors in other cell types, such as proximal tubular cells and cardiac myocytes.<sup>21,22</sup> This shared susceptibility to angiotensin II and AT1 receptor-mediated apoptosis among  $\beta$ -cells, proximal tubular epithelial cells, cardiac myocytes, and endothelial cells may explain the constellation of accelerated nephropathy, coronary artery disease, and cardiomyopathy that is frequently observed in patients with NIDDM. Presumably, these patients overexpress the AT1 receptor, or have heightened signaling through it, as well as increased levels of proteins involved in the apoptosis pathway.<sup>10</sup>

Angiotensin II may also induce apoptosis of pancreatic  $\beta$ -cells indirectly. As mentioned above, the concentration of free fatty acids is elevated in starvation and in the absence of insulin. By creating insulin resistance, angiotensin II can also promote islet cell apoptosis by increasing the plasma concentration of free fatty acids.<sup>23</sup>

## DIABETIC COMPLICATIONS

How activation of ACE might lead to atherosclerosis ("macroangiopathy") was addressed

previously.<sup>5</sup> Here, we focus on the microangiopathic complications of diabetes: nephropathy, neuropathy, and retinopathy.

### *Nephropathy*

One of the earliest stages of diabetic nephropathy is renal hypertrophy, in which the glomerular filtration rate (GFR) is elevated by as much as 50%.<sup>24</sup> It is now generally accepted that angiotensin II is likely to initiate both compensatory renal growth after contralateral nephrectomy, as well as diabetic renal hypertrophy. As discussed below, activation of ACE within the kidney may be the trigger for compensatory renal hypertrophy. Angiotensin II, as "renotropin," mediates glomerulotubular balance. Excessive activation of renal ACE by the osmotic effect of hyperglycemia leads to renal apoptosis, increased work by the remaining nephrons, and hence progression of chronic renal failure.

The kidneys receive 25% of the cardiac output. They perform chemical work on the blood, just as the heart performs mechanical work. Like the heart, it is critical that the kidney's workload (renal blood flow in the case of the kidney; preload in the case of the heart) not exceed its ability to perform the work, or the organism will die. If filtration at the glomerulus exceeds the capacity of the proximal and distal tubule downstream to reabsorb sodium and water, then the organism will become volume-depleted within a few heartbeats.

When the GFR of an individual nephron (the single nephron GFR) remains elevated, the nephron undergoes hypertrophy. This is referred to as glomerulotubular balance. Most of the hypertrophy occurs in the proximal tubule, which performs 85% of the tubular reabsorptive work in each nephron.<sup>25</sup>

The mechanism of glomerulotubular balance at the single nephron level can be better understood by examining compensatory renal growth, since the latter is a special case of the former. In compensatory renal growth after contralateral nephrectomy, all of the nephrons of the remaining kidney enlarge simultaneously. The renal cortex visibly increases in thickness, since it is where the proximal tubules are located. The more distal segments of the nephron, located in the medulla of the kidney, remain the same size. After a week, in

animal models, the remaining kidney is 25% larger.<sup>25</sup>

Biochemical evidence of growth is apparent within 5 min of removing the contralateral kidney.<sup>26</sup> The pattern of immediate-early gene expression 30 min after uninephrectomy,<sup>27</sup> and the increase in renal cortical blood velocity seen within 5 min,<sup>28</sup> strongly suggest that the triggers for renal hypertrophy (the “renotropins”) are angiotensin II and norepinephrine, as in myocardial hypertrophy.<sup>29</sup>

When more than 50% of the total renal mass is removed, either surgically or because of an ongoing disease process such as a glomerulonephritis, the remaining renal parenchyma shrinks. At end-stage, kidneys may measure only 6–8 cm in length by ultrasound, versus 12–14 cm in healthy kidneys. Most of the loss of parenchyma occurs in the cortex: Glomeruli become fibrotic (“sclerosed”) or disappear altogether, as do proximal tubules.

Since this process is gradual and symptomless (i.e., without any systemic inflammatory symptoms such as fever or rigors), it must occur by apoptosis rather than necrosis. At the single nephron level, an ongoing growth signal results in apoptosis. This may reflect a time-dependent switch in signal transduction pathways for the AT1 receptor, from stimulating growth to apoptosis.<sup>21</sup> This is clearly a modern restatement of Bricker’s “remnant nephron” hypothesis.<sup>30</sup>

The duplicated form of ACE is highly expressed in the brush border membrane (BBM) of the proximal tubule, precisely the nephron segment that undergoes the bulk of compensatory renal hypertrophy, as well as apoptosis in progressive renal failure. ACE is also expressed in the afferent arteriole and the glomerular capillary, but in a much smaller amount than in the proximal tubular BBM.

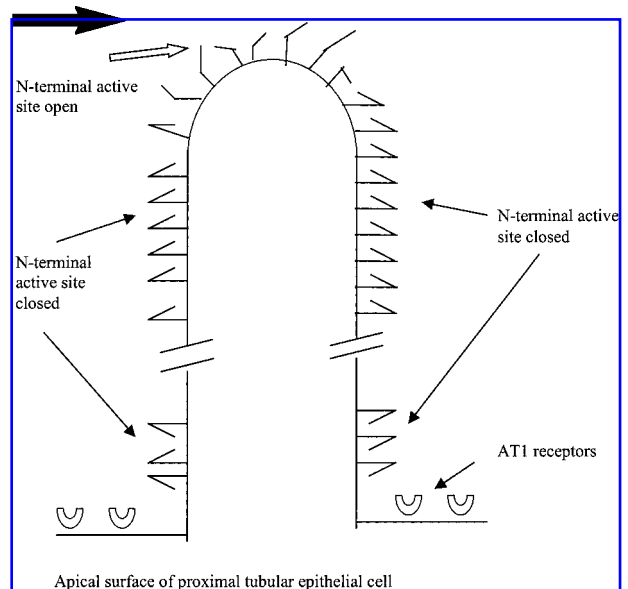
Blood flow in the glomerular capillary is not damped by transit across the glomerular basement membrane.<sup>31,32</sup> Flow in the early proximal tubular lumen remains pulsatile and with similar pressure as in the glomerular capillary ( $P_{GC}$ ). With bulk reabsorption of  $\text{Na}^+$  and water, the flow of tubular filtrate becomes constant by the end of the proximal tubule, and pressure decreases by a factor of 2.<sup>33</sup>

If the duplicated form of ACE (“somatic” ACE) is indeed a mechanosensor, then its lo-

cation in the proximal tubular BBM is ideal for sensing  $P_{GC}$ . Velocity of a fluid is directly proportional to its driving pressure.<sup>5</sup> High-velocity glomerular filtrate is expected to activate ACE molecules at the tip of the BBM<sup>34</sup> (Fig. 1). A mean pressure of 100 mm Hg produces a force of  $\sim 1$  pN acting on a molecule of ACE’s dimensions.<sup>5</sup>  $P_{GC}$  is therefore of the correct magnitude to disrupt van der Waals (hydrophobic) interactions normally keeping the N-terminal active site of ACE occluded by the FQP autoinhibitory tripeptide.<sup>5</sup> The “ACE as mechanosensor” hypothesis explains how  $P_{GC}$  can be transduced directly into biological activity.

This mechanism would clearly explain Brenner’s elegant work elucidating how renal damage is proportional to  $P_{GC}$ , and how both are inversely proportional to nephron number, below a threshold number of about 1 million nephrons in humans.<sup>35</sup>

Because of efficient reabsorption of fluid along the tubule,<sup>33</sup> the relationship of luminal



**FIG. 1.** Proposed mechanism of glomerulotubular balance. A single microvillus of the proximal tubular BBM is depicted; there are dozens of such microvilli on each proximal tubular epithelial cell. The apical membrane of the proximal tubular cell is in the direction of the bottom. Black arrow, direction of glomerular filtrate. Stick figures, individual molecules of ACE (not drawn to scale). Open stick figures, N-terminal active site of ACE opened by mechanical fluid force exerted on distal 15% of microvillar tip<sup>34</sup>; closed stick figures, N-terminal active site of ACE closed because of absence of mechanical fluid force on proximal 85% of microvillar shaft. Cup-shaped figures on apical membrane, AT1 receptors.

fluid velocity to  $P_{GC}$  is less than linear. The damping becomes more severe with distance downstream from the glomerular basement membrane. Nevertheless, increasing  $P_{GC}$  should result in more ACE molecules being activated in the early part of the proximal tubule (Fig. 1). The amount of angiotensin II produced in the BBM of the early proximal tubule will be roughly proportional to  $P_{GC}$ .

This could explain how activation of ACE in the proximal tubular BBM could generate a large local concentration of angiotensin II (Fig. 2), the renin implicated in compensatory renal growth and glomerulotubular balance. Growth-promoting AT1 receptors are located on the apical (luminal) surface of proximal tubular cells<sup>36</sup> as well as their basolateral membrane, and could mediate the growth signal of the renin. After prolonged signaling through AT1 receptors, renal signal transduction may switch from growth promoting G-proteins coupled to PKC to pro-apoptotic tyrosine phosphatases.<sup>21</sup>

Angiotensin II, an octapeptide with a molecular size of ~900 daltons, is small enough to pass freely through the loose intercellular junctions between proximal tubular epithelial cells. Thus, angiotensin II generated within the BBM could rapidly diffuse to basolateral AT1 and AT2 receptors of proximal tubular epithelial cells, as well as those of fibroblasts and other cells in the renal interstitium (Fig. 2). If generated in large amounts, angiotensin II produced

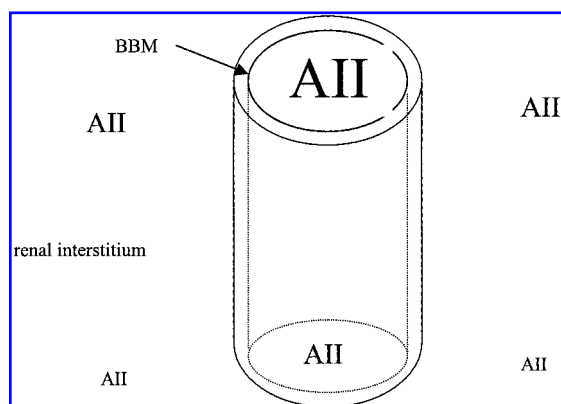
within the proximal tubular BBM could also diffuse upstream against the velocity gradient, and bind to AT1 and AT2 receptors on the surface of renal mesangial cells and epithelial and monocyte cells within Bowman's capsule. Another important source of glomerular angiotensin II is likely to be activated mesangial cells and infiltrating monocytes.

All of the cell types just mentioned respond to angiotensin II: Proximal tubular cells undergo hypertrophy; interstitial fibroblasts synthesize type IV collagen and proliferate; mesangial cells contract, lowering the ultrafiltration coefficient ( $K_f$ ); and monocytes proliferate. High angiotensin II levels in the glomerular mesangium and renal interstitium induce transforming growth factor (TGF)- $\beta$  synthesis,<sup>37</sup> resulting in mesangial and interstitial fibrosis. The latter is highly correlated with progression of kidney disease.<sup>38</sup>

Angiotensin II generated in the BBM is therefore sufficient to produce the hallmark glomerular, tubular, and interstitial changes of progressive renal disease.

Norepinephrine, implicated by fibronectin's expression as an immediate-early gene,<sup>27</sup> may act synergistically with angiotensin II.<sup>39</sup> The other immediate-early genes examined after contralateral nephrectomy had 12-*O*-tetradecanoyl-phorbol-13-acetate-response elements (TREs) in their promoters, so their transcription was consistent with activation of PKC.<sup>27</sup> But fibronectin's promoter lacks TREs, and has only a cyclic AMP-response element, requiring signal transduction by protein kinase A. By analogy to myocardial hypertrophy<sup>29</sup> and liver regeneration,<sup>40</sup> norepinephrine is presumed to be the activator of adenyl cyclase in compensatory renal growth. Elsewhere in the body, angiotensin II and norepinephrine are synergistic: Angiotensin II stimulates synthesis and release of norepinephrine in the peripheral nervous system and the heart.<sup>41-43</sup> The source of renal norepinephrine after contralateral nephrectomy is presumably the sympathetic nerves. The relative unimportance of sympathetic nerves in compensatory renal growth, however, suggests that angiotensin II is the major renotropin.<sup>44,45</sup>

End-stage renal disease is far more common than renal adenocarcinoma, although both are



**FIG. 2.** Angiotensin II (AII) concentration gradient within the renal cortex. An early segment of the renal proximal tubule is shown. The relative concentrations of AII near the BBM and within the renal interstitium are suggested by the size of the type.

associated with the ACE *D/D* genotype.<sup>10</sup> The kidney therefore appears to be far more predisposed to apoptosis than to cancer. The unusual link between the AT1 receptor and apoptosis<sup>21</sup> may help explain this.

### *Heart disease*

Both type 1 and type 2 diabetes carry an increased risk of various forms of heart disease: atherosclerotic coronary artery disease,<sup>46</sup> left ventricular hypertrophy (LVH),<sup>47</sup> and dilated "diabetic" cardiomyopathy.<sup>48</sup>

### *Myocardial Hypertrophy/Dilated Cardiomyopathy.*

Both ventricles are capable of undergoing hypertrophy in the face of increased afterload. The signal for myocardial hypertrophy appears to consist of angiotensin II and norepinephrine.<sup>29</sup> How does the hypothesis that ACE is a mechanotransducer explain the molecular mechanism for initiation of myocardial hypertrophy, and the acceleration of myocardial ischemia by LVH?

The heart compensates for an acute increase in afterload by increasing its force of contraction. In systemic hypertension caused by a reset pressure–natriuresis curve, the carotid sinus senses decreased flow, and acts through a central nervous system reflex to stimulate sympathetic nerves that end on myocardial fibers. The effect of norepinephrine release by sympathetic nerve fibers is to increase the force of contraction; acutely, this involves increased  $\text{Ca}^{2+}$  influx into each fiber.

More forceful contraction in response to increased sympathetic nerve activity results in depletion of ATP stores within myocardial fibers. Adenosine, the breakdown product of ATP, increases intracellularly. Released extracellularly, adenosine stimulates vasodilation of coronary arteries and arterioles. Myocardial blood flow increases. The vessels that perfuse the heart travel within the muscle, and are occluded during systole. The more hypertrophied the myocardium, the greater the occlusive pressure on perforating blood vessels. In LVH, diastolic relaxation of the ventricle is impaired, limiting myocardial perfusion during diastole. Further myocardial ischemia results from LVH, leading to further release of adenosine from ischemic myocardial fibers. The net result is

that blood velocity increases within myocardial arteries, arterioles, and capillaries.

An increase in blood velocity results in increased mechanical shear forces in vessels with laminar as well as turbulent flow. In areas of turbulent flow, the oligopeptide flap that normally binds and inactivates the N-terminal active site of ACE is forced open mechanically, exposing a second active site on the ACE molecule and dramatically increasing local production of angiotensin II and degradation of bradykinin, as well as other peptides.<sup>5</sup>

A positive feedback loop results, since the increase in local angiotensin II concentrations will act synergistically to enhance norepinephrine release and reuptake by sympathetic nerve terminals. This will lead to stronger neural signaling, increased force of contraction, more myocardial ischemia and accumulation of adenosine, increased myocardial blood velocity, further activation of endothelial ACE, etc.

The natural course of disease is for systemic hypertension to lead to LVH, and then to dilated cardiomyopathy (i.e., the syndrome of congestive heart failure). This is the clinical expression of the biochemical pathway set in motion by angiotensin II: first cell growth, via phosphorylation of c-myc; after prolonged signaling with high levels of angiotensin II, cell suicide (apoptosis) perhaps via dephosphorylation of c-myc.<sup>10</sup> By increasing plasma osmolality ( $\mu$ ), the hyperglycemia of diabetes accelerates this process by amplifying the activation of endothelial ACE at any given blood velocity. Diabetes increases the gain of the system by increasing  $\mu$ .

### *Neuropathy*

The vasa nervorum are the key to the survival and proper functioning of peripheral nerves, although their regulation is complex. Endothelial ACE is hypothesized to be activated by turbulent flow in the vasa nervorum. As discussed above, activation of endothelial ACE should be enhanced by the increased osmolality ( $\mu$ ) due to hyperglycemia in diabetic patients. High angiotensin II levels in diabetic vasa nervorum are therefore expected to induce vasoconstriction. The latter is usually observed in animal models of diabetes and in patients.<sup>49,50</sup>

Severe vasoconstriction (blood flow  $<5$  mL/100 g/min) is required for neural death by necrosis.<sup>49</sup> But, at least in the central nervous system, less severe vasoconstriction, which produces ischemia, can lead to death by apoptosis.<sup>51</sup> In the central nervous system, ischemia results in replacement of anti-apoptotic AT1 receptors by pro-apoptotic AT2 receptors. Ongoing production of angiotensin II by activated ACE molecules would supply the elevated angiotensin II concentrations that trigger apoptosis.

If this mechanism holds for peripheral nerves as well, then it would explain our observation that overactivity of ACE (the *D/D* genotype) was generally associated with neuropathy in NIDDM.<sup>10</sup>

### *Eye disease*

Three forms of eye disease are more common among patients with type 1 or type 2 diabetes: proliferative retinopathy, glaucoma, and cataracts. Recently, the eye was found to contain a tissue renin-angiotensin system.<sup>52,53</sup>

*Retinopathy.* We observed retinopathy in type 1 and 2 diabetes to be associated with ACE overactivity.<sup>10</sup> Activation of endothelial ACE by hyperglycemia, as described above, will produce more angiotensin II within the eye. This will be exacerbated by systemic hypertension, a known risk factor for both diabetic retinopathy and nephropathy (microangiopathy).

The retina is extremely sensitive to vasoconstriction and ischemia, and displays exuberant neovascularization in response to hypoxia. Angiotensin II is a potent angiogenesis factor in its own right,<sup>54</sup> and it stimulates the production of vascular endothelial growth factor (VEGF), already implicated in diabetic proliferative retinopathy.<sup>55,56</sup> Angiotensin II, via stimulation of PKC isoform  $\beta$ , amplifies the effect of VEGF to induce endothelial cell proliferation.<sup>57</sup>

*Glaucoma.* Glaucoma is approximately twice as common among diabetic patients than within the population at large. Glaucoma is also linked epidemiologically to hypertension, and has an incidence approximately twice as great among African Americans as among American Caucasians. These epidemiologic observations are

consistent with our findings that the ACE *D/D* genotype is associated with glaucoma (OR = 1.6) and hypertension (OR = 1.2), and that the frequency of the ACE *D/D* genotype is higher among people of African descent.<sup>10</sup>

The duplicated form of ACE is normally present in the ciliary body of the eye, where the anterior fluid crosses into the posterior chamber of the eye.<sup>58</sup> Although the velocity is small, the viscosity of the vitreous is high, so flow from the anterior chamber through the ciliary epithelium to the posterior chamber may generate sufficient shear stress to open the FQP flap occluding the N-terminal active site of ACE.<sup>5</sup> Activation of endothelial cell ACE by systemic hypertension, with which glaucoma is associated, will also elevate angiotensin II concentration within the vitreous.<sup>52</sup>

The consequence of ACE activation is to decrease the flow across the ciliary epithelium and increase intraocular pressure.<sup>59-63</sup> The mechanism of flow is still not well understood. It is not simple filtration, but appears to involve active transcytosis by mesangial-like cells located within the trabecular meshwork. In the short term, angiotensin II may stimulate contraction of mesangial-like cells present within the trabecular meshwork to decrease their permeability (" $K_f$ " by analogy with the renal glomerulus). In the long term, angiotensin II stimulates TGF- $\beta$ 1 which may lead to apoptosis of ciliary epithelial cells and fibrosis. This is the pathology seen in primary open-angle glaucoma.<sup>64</sup>

Myocilin, a gene associated with hereditary glaucoma,<sup>65</sup> has several AP-1 sites in its promoter. Transcription of the myocilin gene could therefore be activated by angiotensin II, signaling through PKC.

*Cataracts.* Cataracts are more common among patients with diabetes. We observed an elevated OR for the ACE *D/D* genotype and cataracts among diabetic as well as nondiabetic patients.<sup>10</sup>

Although there has been a great deal of interest in aldose reductase in the rat model of cataracts, humans (like mice) have low levels of this enzyme.<sup>66</sup> Rather, TGF- $\beta$  seems best able to mimic human cataracts in a mouse model.<sup>64</sup> Expression of TGF- $\beta$  is induced specifically by



angiotensin II.<sup>37</sup> Thus, cataracts may also be seen as a result of overactivity of endothelial ACE due to the increased osmolality of diabetic hyperglycemia. Angiotensin II produced on capillary endothelial cell membranes accumulates in the vitreous.<sup>52</sup> Age-related cataracts may have a similar mechanism, although their progression is slower than in diabetes because of the absence of hyperglycemia and lower plasma osmolality in normal aging.

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Address reprint requests to:

*D.W. Moskowitz, M.D.*

*Chairman and CMO*

*Genomed, Inc.*

*909 S. Taylor Avenue*

*St. Louis, MO 63110*

*E-mail: dwmoskowitz@genomedics.com*