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REVIEW

Role of Endothelin in Diabetic Vascular Complications

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Endothelin-1 (ET-1), a 21 amino acid peptide originally purified from conditioned medium of cultures of porcine aortic endothelial cells, is recognized as a product of many other cells as well. It is now known that there are three endothelin genes in the human genome (*ET-1*, *ET-2*, and *ET-3*). ET-1 and ET-2 are both strong vasoconstrictors, whereas ET-3 is a potentially weaker vasoconstrictor than the other two isoforms. Besides being the most potent vasoconstrictor yet known, ET-1 also acts as a mitogen on the vascular smooth muscle, and, thus, it may play a role in the development of vascular diseases. It is well known that accelerated angiopathy is a major complication in diabetes mellitus. As generalized endothelial cell damage is thought to occur in diabetic patients, ET-1, being released from the damaged endothelial cells, is able to make contact with the underlying vascular smooth muscle cells and thus could be one important cause of diabetic angiopathy. This article summarizes the reported literature of the role of ET-1 in the development of diabetic complications, with particular focus on the possible role of ET-1 in mediating the effects of angiotensin-converting enzyme inhibitors.

Key Words: Endothelin; diabetic vascular complications; ACE inhibitors.

Biology of Endothelins

In 1988, Yanagisawa et al. *(1)* isolated a 21 amino acid vasospastic peptide from the supernatant of cultured porcine endothelial cells and called this peptide endothelin (ET). As far as a pressor agent is concerned, ET is found to be the most potent vasoconstrictor yet known. There are three distinct ET genes that encode different mature ET sequences, designated *ET-1*, *ET-2*, and *ET-3 (2)*. Human ET-1 mRNA encodes a 212 amino acid prepropeptide that is cleaved by one or more dibasic pair-specific endopeptidases to yield 38 amino acid "big ET-1." Big ET-1 is converted into mature 21 amino acid ET-1 by a putative ET-converting enzyme (ECE). It is now known that there is not a single ECE but a group of related proteins that have different preferences for big ET-1, big ET-2, and big ET-3 as substrates. At present, two distinct ECEs (ECE-1 and ECE-2) have been cloned and sequenced *(3,4)*. ECE-1 is believed to be predominantly expressed on the cell surface, whereas ECE-2 is presumably located intracellularly in close proximity to the *trans*-Golgi network. Four different isoforms of human ECE-1 (ECE-1a *[5]*, ECE-1b *[6]*, ECE-1c *[7]*, and ECE-1d *[8]*) have been identified. They differ only in their short N-terminal cytosolic regions and are derived from a single gene through the use of alternative promoters. In addition, they have distinct tissue distribution and intracellular localization. ECE-1a, ECE-1c, and ECE-1d are all located at the cell surface, whereas ECE-1b is targeted to an intracellular compartment and shows significant colocalization with a marker protein for the *trans*-Golgi network *(7)*.

The three isoforms of mature peptides (ET-1, ET-2, ET-3) are distributed widely but in different proportions throughout many tissues. For instance, ET-1 is predominant in endothelial cells, whereas all three types of ET are detected in kidney and jejunum *(9,10)*. Fast protein liquid chromatography reveals that the major component of ET in human hypothalamus and brain stem is ET-1, whereas the ET in human pituitary gland is mainly ET-3 *(11)*. However, a similar but reverse finding was noted in the brain of rat *(12)*. The diverse and uneven distribution of ET isoforms suggests that ET has multiple functions not only in the cardiovascular system but also in the nonvascular system. In 1990, Arai et al. *(13)* have cloned a bovine ET receptor (named ET_A) that has a high affinity to ET-1 and ET-2 but a low affinity to ET-3. A second population of receptors (named ET_B) was also cloned in 1990, by Sakurai et al. *(14)*, in which all three isoforms are equipotent. In 1993, Karne et al. *(15)* cloned the third receptor (ET_C), which has increased specificity for ET-3. ET_A receptors are found in vascular smooth muscle cells and mediate vasoconstriction and cell proliferation. ET_B receptors are found in endothelial cells and mediate vasodilation via the release of nitric oxide. A variety of additional functions has been attributed to ET_B receptors; however, in certain instances,

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they may even elicit vessel contraction *(16)*. Moreover, ET_B receptors also act as clearance receptors and initiate a positive autocrine loop by which ET-1 regulates expression of its own gene *(17)*. Coupling of activated receptors to effector systems generates the second messengers, which include calcium, inositol phosphates, and diacylglycerols and are ultimately responsible for biologic effects *(9)*.

Originally identified as a product of endothelial cells and acting as the most potent vasoconstrictor, ET is now known to be a product of many other cells, including osteoblasts *(18)*, and has diverse polyfunctional roles *(19)*.

Role of ET in Diabetic Vascular Complications

There is evidence that impaired autoregulation of blood flow is involved in the pathogenesis of diabetic microangiopathy, and microvascular disease is an important cause of morbidity in diabetes *(20)*. The vascular endothelium plays a central role in the regulation of vascular tone, and ET-1, a potent endothelium-derived vasoconstrictor substance, is regarded to contribute significantly to the maintenance of basal vascular tone *(21)*. Recently Nugent et al. *(22)* demonstrated that there was no reduction in forearm blood flow in response to ET-1 in patients with noninsulin-dependent diabetes mellitus (NIDDM). This impaired vasoconstriction to ET-1 could result in hyperperfusion and subsequent microvascular damage. This in turn may cause damaged production and release of substances such as ET-1 synthesized by endothelial cells, altered barrier function and decreased antithrombotic activity of the endothelium which are well known events noted in diabetic patients. As a result of the impaired endothelial barrier, a number of endothelial-producing substances including ET, which previously could not pass the intact endothelial barrier, are able to get in contact with and exert their effects on the underlying vascular smooth muscle cells. As a result of this pathological process, diabetic subjects are prone to vascular ischemia, atherosclerosis, hypertension, and various macrovascular diseases *(23)*.

Diabetes affects the microcirculation, the large arteries, and occasionally the large and small veins, by inducing vessel wall sclerosis. The ability of the diabetic person's circulation to distribute blood is affected, especially during increased blood flow. In most tissues, this causes no serious burden, but three tissues are usually susceptible to disturbance: the retina, renal cortex, and peripheral nerves *(20)*.

Role of Local ET-1 in Diabetic Retinopathy

Retinal vascular autoregulation is defined as the ability of the blood vessels to keep blood flow constant under varying perfusion pressure *(24)* in order to match it to tissue oxygen and metabolic requirements *(25)*. The failure of autoregulation is an important and often early feature of diabetic retinopathy *(26)*. Because human retina vessels lack extrinsic innervation, retinal vessel caliber and local blood flow are normally regulated by nonnervous mechanisms intrinsic to the retina *(27)*. Pericytes are intramural cells that surround the endothelial cells in capillary and postcapillary venules *(28)*. There is now a considerable body of evidence to suggest that retinal pericytes are the main regulators of vascular tone in the retinal capillaries because they contain components of contractile proteins similar to vascular smooth muscle cells *(29)*. Our colleagues, Takahashi et al. *(30)*, have previously shown that retinal endothelial cells can secrete ET-1, and corresponding pericytes bear receptors for this peptide, suggesting the presence of a specific interactive system for the regulation of retinal capillary blood flow in a circulation that shows no evidence of extrinsic innervation. Furthermore, ET-1 has been shown to cause vasoconstriction of retinal vessels *(31)* as well as to have mitogenic effects on retinal pericytes *(32)*. Hence, alterations in the pericyte-ET interaction may have a role causing early hemodynamic and histopathologic abnormalities found in diabetic retinopathy *(28)*.

Ogata et al. *(33)* have demonstrated that the immunoreactive vitreous ET levels in NIDDM patients with proliferative retinopathy were significantly lower than those in nondiabetic subjects. This decrease in vitreous ET in diabetic patients may be owing to hyperglycemia *per se*, or as a consequence of insulin deficiency or insulin resistance, which are common features noted in NIDDM patients *(34)*. Two recent studies have shown that vascular endothelium maintained in vitro under conditions of hyperglycemia simulating diabetes releases less ET-1 than under normoglycemic conditions *(35,36)*. However, Metsärinne et al. *(37)* and by Yamauchi et al. *(38)* noted, respectively, that high glucose either has no effect or has an opposite effect. In addition, it has been shown that insulin can stimulate ET-1 secretion both in vivo *(39)* and in vitro (cultured endothelial and vascular smooth muscle cells) *(40,41)*. Moreover, not only does insulin stimulate ET-1 gene expression but it also increases the number of ET-1 receptor-binding sites *(42)*. We have previously demonstrated that plasma concentrations of ET in dexamethasone-treated rats were significantly higher than those in controls, whereas plasma concentrations of ET in streptozotocin-treated rats and rats treated with both dexamethasone and streptozotocin were undetectable *(43)*. These findings suggest that endogenous insulin may have a role in the regulation of plasma ET levels. Recently, Temple et al. *(34)* have shown that most NIDDM patients are in fact insulin deficient; it is not surprising to find that there is a lower vitreous ET level in NIDDM patients. Even though Frank et al. *(42)* did not show any effect of glucose on the number of ET-1 receptors as well as gene expression of ET-1 in bovine vascular smooth muscle cells, other researchers have shown that hyperglycemia itself can reduce the effect of ET-1 and the activity of diacylglycerol and protein kinase C (PKC) in cultured calf retinal pericytes *(44)*. Similarly, Bursell et al. *(45)* also demonstrated a significant blunted response of

the retinal circulation to intravitreal injection of ET-1 in diabetic rats when compared with nondiabetic rats. Because ET-1 is known to have mitogenic effects on retinal pericytes *(32)*, a decrease in ET-1 production or action may influence pericyte replication, maintenance, and health and may account, at least in part, for the loss of pericytes, which is a feature of earliest histologic change in diabetic retinopathy long before any clinically visible lesions develop.

In summary, the reduced local ET concentration in the eyes of diabetic patients together with the resistance of ET action on the corresponding pericytes could be a direct cause for dilatation and increase in blood flow of the retinal capillaries, loss of normal ocular autoregulation, formation of retinal microaneurysms, as well as leakage of fluid —typical features noted by histologic studies in diabetic retinopathy *(24)*. On the contrary, Chakrabarti et al. *(46)* demonstrated that retinas from chronic diabetic BB/W rats (6 mo) showed an increase in ET-1, ET-3, ET_A, and ET_B mRNA expressions when compared with those from control rats. Similar results were also noted by using immunohistochemical methods *(47)*. Finally, increased ocular and retina tissue levels of ET-1 in diabetic rats have also been reported by Chakravarthy et al. *(48)* as well as by Takagi et al. *(49)*. All these findings suggest that ETs also may be involved in the pathogenesis of more advanced diabetic retinopathy such as capillary occlusion and subsequent neovascularization.

Role of Local ET-1 in Diabetic Nephropathy

Diabetes causes local loss of microvascular autoregulation in the kidneys *(20)*. Similar to what occurs in the retina, a decrease in renal production of ET-1 or a reduction in ET-1 action in mesangial cells, which are smooth muscle-like cells, may account for the disturbed microvascular autoregulation that occurs in the early course of diabetic nephropathy. Early stages of human and experimental insulin-dependent diabetes mellitus (IDDM) as well as NIDDM are characterized by an elevation of glomerular filtration rate (GFR) *(50,51)*. Lam et al. *(52)* are the first to demonstrate that 24-h urinary ET-1 excretions in recent-onset type 2 diabetes mellitus patients are significantly lower than that of normal subjects. Similar results were confirmed by De-Mattia et al. *(53)*, who also demonstrated a markedly reduced 24-h urinary ET-1 excretion in NIDDM patients with or without microalbuminuria. Interestingly, the degree of ET-1 reduction in urine was very close between the two studies. Furthermore, Shin et al. *(54)* have shown that moderate hyperglycemia in streptozotocin-induced diabetic rats is associated with a decrease in renal ET-1 content and gene expression. Taken together, these studies suggest that an early decrease in renal ET-1 production may occur in diabetes mellitus. Recently, Awazu et al. *(55)* reported that diabetes mellitus is characterized by downregulation of the glomerular ET-1 receptor, which can be reversed by diminishing the abnormally enhanced protein kinase C activity. In addition, Hurst et al. *(56)* have demonstrated a decreased responsiveness to ET-1 of glomerular mesangial cells isolated from streptozotocin-administered rats in high-glucose medium. We suggest that during the early phase of diabetes mellitus, these factors may cause intraglomerular hypertension and thus may contribute to the elevation of GFR observed in diabetic subjects, as well as to the formation of intrarenal microaneurysms observed in patients with early diabetic nephropathy, probably via a mechanism similar to that for diabetic retinopathy (Fig. 1).

It has been proposed that intraglomerular hyperfunction may play a pivotal part in the initiation and progression of diabetic glomerulopathy *(57)*. Although De-Mattia et al. *(53)* did not demonstrate any statistically significant difference in urinary ET-1 excretion between normoalbuminuric NIDDM patients and their microalbuminuric counterparts, the latter group of patients did show a higher urinary ET-1 excretion. Interestingly, Lee et al. *(58)* have reported that there is an increase in 24-h urinary ET-1 excretion in NIDDM patients with proteinuria, which represents a more advanced stage of diabetic nephropathy. This is not surprising because Orisio et al. *(59)* noted that renal ET-1 gene expression increases as the disease progresses to renal insufficiency and urinary ET-1 excretion has also been reported by Zoccali et al. *(60)* to be increased in patients with impaired renal function. Besides being a potent mitogen for mesangial cells *(61)*, ET-1 stimulates the extracellular matrix protein gene expression *(62)*, as well as causes a dose-dependent reduction in renal blood flow and GFR *(63)*. Hence, the increase in renal ET-1 production found in diabetic patients of advancing stage may further deteriorate kidney function and lead finally to the uremic stage, requiring hemodialysis.

Role of Local ET-1 in Diabetic Neuropathy

Although it is generally believed that ischemia and hypoxia may be important in the pathogenesis of some of the neuropathic complications that occur in diabetes *(64)*, evidence for a microcirculatory role in diabetic peripheral nerve damage is not as conclusive as for the kidney and retina *(20)*. It is controversial whether early conduction abnormalities of polyneuropathy in diabetic axons can be accounted for by reduction in nerve blood flow, observed by some *(65,66)* but not all investigators *(67,68)*, or decreased perfusion of dorsal root ganglia *(69)*. It is well known that the longest peripheral nerves are typically the most affected and that nerve damage can be produced by a disturbance in local pressure-flow relationships combined with epineurial mechanical constraint *(20)*. Nukada *(70,71)* have demonstrated that diabetic axons are particularly sensitive to ischemia. Kihara and Low *(72)* have shown that diabetic vasa nervorum, the microvessels that supply nerve trunks, may account for this susceptibility because these microvessels

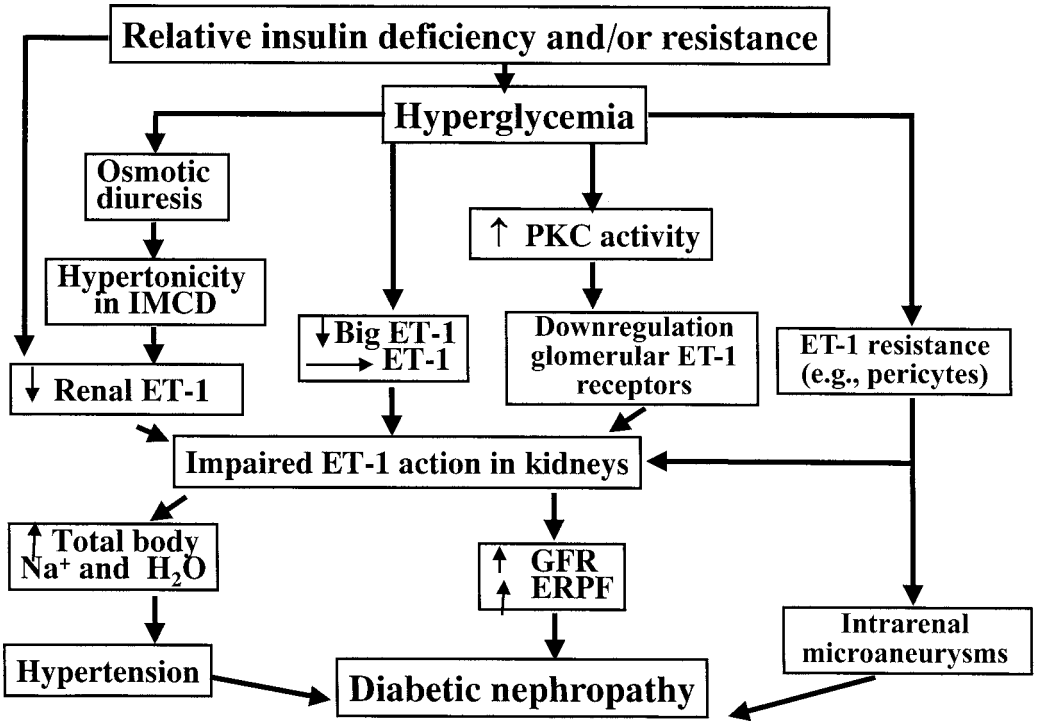

Fig. 1. Proposed mechanisms of pathogenesis of diabetic nephropathy. IMCD, inner medullary collecting duct; ERPF, effective renal plasma flow; GFR, glomerular filtration rate. (Adapted with permission from ref. *52*.)

have been shown to have impaired nitric oxide vasodilation but preserved responsiveness to ET-1 vasoconstriction. This is further supported by Zochodne et al. *(73)*, who found that vasa nervorum are highly susceptible to vasoconstriction from the local application of ET-1. They also demonstrated that diabetic nerves are selectively susceptible to ET-mediated ischemia with resultant severe axonal degeneration *(74)*. Hence, ET-1, being a potent vasoconstrictor of vasa nervorum, through elevated circulating levels, may promote microangiopathy in human diabetes *(75)*. The effect of ET-1 in causing neurovascular dysfunction in experimental diabetic neuropathy may be mediated via ET_A *(76,77)* or ET_B receptors *(78,79)*. In addition, free radicals and the interaction of ET-1 with the renin-angiotensin system may also have a role in altering microvascular function in diabetes *(78)*.

It is generally believed that elevated circulating ET-1 levels may reflect local overproduction of peptide from damaged endothelial cells with plasma "overflow" *(21)*. Francavilla et al. *(80)* have shown that circulating ET-1 levels in diabetic men with erectile dysfunction are elevated when compared with normal men, and that plasma ET-1 levels in cavernous body blood are comparable with levels in peripheral vein blood. Furthermore, the finding of increased ET-1 concentrations in the peripheral vein as well as in the cavernous blood of the same magnitude in diabetic men with erectile dysfunction is indicative of endothelial dysfunction in diabetic men with erectile failure. Increased ET-1 release from altered endothelial cells in cavernous tissues might contribute, at least in part, to erectile dysfunction through its direct contracting effect on corporeal smooth muscle acting through a receptor-mediated effect *(81)*.

Role of Circulatory ET-1 in Diabetic Vascular Complications: Biologic Curiosity or Pathologic Importance?

Increased levels of circulating ET-1 in diabetic patients (IDDM and NIDDM) were first reported by Takahashi et al. *(82)* and subsequently confirmed by other researchers *(53,83)*. In our study, the elevation of ET cannot be explained by secondary changes in blood pressure or renal disease and do not correlate with the presence of diabetic retinopathy, duration of diabetes mellitus, fasting blood glucose, or serum fructosamine. Kawamura et al. *(84)* have shown that circulating ET-1 levels are elevated in NIDDM patients with retinopathy, regardless of the stage of the complications (simple retinopathy or proliferative retinopathy). Similarly, De Mattia et al. showed that circulating ET-1 levels are elevated in NIDDM patients, regardless of the presence or absence of microalbuminuria *(53)*, and Donatelli et al. *(85)* also demonstrated higher plasma ET-1 levels in NIDDM patients, regardless of the presence or absence of macroangiopathy. Taken together, these three studies indicate that an increase in plasma ET-1 concentrations is an early phenomenon rather than a result of advanced stage of diabetes mellitus. Hence, we and others have concluded that ET-1 might be a marker of endothelial

dysfunction. However, some researchers have reported similar but slightly different results. For example, Letizia et al. *(86)* demonstrated that plasma ET-1 concentrations are elevated in NIDDM patients, especially in those with preproliferative retinopathy, but not in IDDM patients and healthly subjects. Furthermore, Laurenti et al. *(87)* showed that plasma ET-1 levels are significantly higher in NIDDM patients with retinopathy than those without retinopathy, and that the ET-1 levels in plasma are directly correlated with degree of retinopathy. They suggest that the increased plasma levels of ET-1 could contribute to the development of retinopathy or, more likely, represent a marker of this diabetes-related complication. Haak et al. *(88)* have found that diabetic patients have higher plasma ET-1 concentrations than normal subjects. However, contrary to our findings, Haak et al. *(88)* demonstrated that the elevation of plasma ET-1 appears to depend on the duration of diabetes, and to correlate with the occurrence of arterial hypertension and reduced renal function as diabetic complications. Hence, they suggest that in diabetes mellitus, elevated ET levels act at least as a marker or may even play a pathophysiologic role in the development of hypertension and its sequelae, as well as in the development of diabetic vascular complications.

By contrast, Bertello et al. *(89)* failed to demonstrate any difference in plasma ET-1 levels in NIDDM patients, whether or not they have angiopathy. A similar finding was also noted by Kamoi et al. *(90)* for ET-1, except that they also measured plasma big ET-1 and found it markedly elevated only in patients with microangiopathy. The reasons for the difference in plasma ET-1 levels reported among various laboratories could be owing to a difference in the assays of ET-1 using polyclonal and monoclonal antibodies of various origin as well as a difference in extraction techniques *(21)*. Interestingly, Kamoi et al.'s *(90)* finding is in complete agreement with that demonstrated previously by Tsunoda et al. *(91)*. These two studies indicate that the conversion of big ET-1 to ET-1 is reduced in diabetic subjects associated with diabetic microangiopathy, which may be the effect rather than the cause of endothelial dysfunction. The significance of this elevated "big" ET-1 in diabetic patients is not known. However, Hoffman et al. *(92)* have demonstrated that big ET-1 has an opposite effect on the kidney than ET-1. In addition, they also demonstrated that big ET-1 may also competitively inhibit binding of ET-1 to its receptor. These findings suggest that big ET-1 may have a role in the development of diabetic complications.

Effects of Angiotensin-Converting Enzyme Inhibitors on Diabetic Complications: Does ET Play a Role?

It is well known that blood pressure is an important risk factor for the development of diabetic complications *(93, 94)*. Antihypertensive therapy, especially angiotensin-converting enzyme (ACE) inhibitors, has been shown to manifest a greater antiproteinuric effect than other classes of antihypertensive agents, despite similar antihypertensive actions *(95)*. They can slow the progression of nephropathy, even in patients with early signs of renal disease without hypertension *(96,97)*. Recently, ACE inhibitors were also shown to decrease the progression of retinopathy in normotensive type 1 diabetic patients with little or no nephropathy *(98)*. By complex interactions with various endocrine systems such as the renin-angiotensin system, aldosterone, and atrial natriuretic factor (ANP), ET may modulate cardiovascular and renal function indirectly. Yoshida and Nakamura *(99)* have shown that captopril inhibits the stimulated release of ET from cultured human endothelial cells. We have also demonstrated that short-term administration of captopril in normal subjects decreases the 24-h urinary ET-1 excretion *(100)*. In addition, Ferri et al. *(101)* have demonstrated that plasma ET-1 levels are decreased significantly after captopril therapy. Thus, from the results of our studies and others, we speculate that ACE inhibitors may exert their effects in humans not only by blocking the renin-angiotensin and kinin systems, but also by inhibiting the production or release of ET *(100)*. Furthermore, in a recent article *(102)*, we proposed a possible role of ET in mediating the hypoglycemic effect of ACE inhibitors (Fig. 2). In fact, the influence of ET-1 secretion by ACE inhibitors is not unique for captopril. Fukui et al. *(103)* have demonstrated that enalapril can also attenuate the increases in ET-1 mRNA levels observed in the glomeruli of diabetic rats but has no effect on increased mRNA levels of tumor necrosis factor-α, platelet-derived growth factor (PDGF)-B chain (PDG-B), transforming growth factor-β, and bovine fibroblast growth factor. Moreover, Hocher et al. *(104)* have also shown that trandolapril, although it has no effect on plasma ET-1 levels, can reduce urinary ET-1 excretion in diabetic streptozotocin rats.

Compared with nondiabetic subjects, diabetic patients are prone to develop cardiovascular diseases such as hypertension, coronary artery disease, myocardial infarction, congestive heart failure (CHF), atherosclerosis, peripheral arterial occlusive disease, and stroke *(105)*. It is well known that ACE inhibitors can improve symptoms and hemodynamics in patients suffering from these heart diseases, including those suffering from severe and chronic CHF; however, although several reports have failed to detect an effect of ACE inhibitors on plasma ET-1 levels in CHF patients *(106,107)*. Hence, ACE inhibitors may affect the actions of ET in humans without necessarily causing changes in plasma ET immunoreactive concentrations. Recently, Galatius-Jenson et al. *(108)* demonstrated one ACE inhibitor, fosinopril, to be effective in normalizing ET-1 levels in patients with chronic CHF. They have put forth the interesting hypothesis that the unique chemical structure of fosinopril, with "phosphinic" as its active group, might interact with the active site of the ECE, thereby explaining

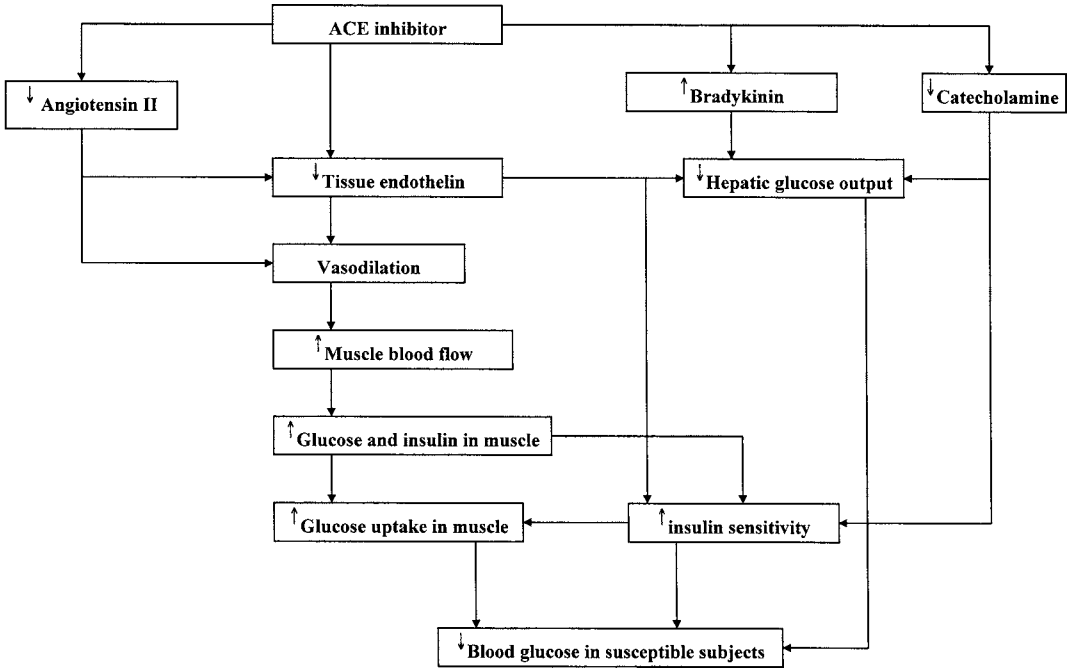

Fig. 2. Proposed mechanism for the ACE inhibitor–related hypoglycemia in susceptible subjects. (Adapted with permission from ref. *102*.)

fosinopril's demonstrated effect on plasma ET levels. Since the elevated plasma levels of ET found in patients with heart failure have been shown to result in vasoconstriction *(109)*, Monge *(110)* suggests that the ability of fosinopril to lower plasma ET levels may point to a superior role of fosinopril compared with other ACE inhibitors in CHF patients.

Conclusion

More and more evidence suggests that ET may have a role in diabetic vascular complications. A decrease in secretion or action of local ET may have a role in the pathogenesis of early diabetic vascular complications, whereas an increase in ET release from the damaged endothelium, either locally or systematically, may be an important mediator of advanced diabetic vascular complications. The appropriate use of ACE inhibitors may provide benefit beyond blood pressure control in patients with diabetic vascular complications.

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