Critical Review

Angiotensin Converting Enzyme 2: A New Important Player in the Regulation of Glycemia

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Abstract

In spite of the novel antidiabetic drugs available on the market, type 2 diabetes mellitus (T2DM) affects nearly 25 million people in the USA and causes about 5% of all deaths globally each year. Given the rate and proportion by which T2DM is affecting human beings, it is indispensable to identify new therapeutic targets that can control the disease. Recent preclinical and clinical studies suggest that attenuating the activity of the renin–angiotensin system (RAS) could improve glycemia in diabetic patients. Angiotensin-converting enzyme 2 (ACE2) counteracts RAS overactivity by degrading angiotensin-II (Ang-II), a vasoconstrictor, to Ang-(1–7) which is a vasodilator. A decrease in ACE2 and an increase in A disintegrin and metalloproteinase (ADAM17)-mediated shedding activity have been observed with the progression of T2DM, suggesting the importance of this mechanism in the disease. Indeed, restoration of ACE2 improves glycemia in db/db and Ang-II-infused mice. The beneficial effects of ACE2 can be attributed to reduced oxidative stress and ADAM17 expression in the islets of Langerhans in addition to the improvement of blood flow to the β-cells. The advantage of ACE2 over other RAS blockers is that ACE2 not only counteracts the negative effects of Ang-II but also increases Ang-(1–7)/Mas receptor (MasR) [a receptor through which Ang-(1–7) produces its actions] signaling in the cells. Increased Ang-(1–7)/MasR signaling has been reported to improve insulin sensitivity and glycemia in diabetic animals. Altogether, ACE2/Ang-(1–7)/MasR axis of the RAS appears to be protective in T2DM and strategies to restore ACE2 levels in the disease seem to be a promising therapy for Ang-II-mediated T2DM. © 2013 IUBMB Life, 65(9):731–738, 2013

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Introduction

Type 2 diabetes mellitus (T2DM) is reaching an epidemic proportion affecting 250 million people worldwide. Given the rate of increase in number of diabetic patients, the World Health Organization (W.H.O.) speculates that nearly 500 million people would suffer from T2DM in 2030 (W.H.O. diabetes factsheet September 2012). To avoid such enormous rise in the prevalence of T2DM, it becomes imperative to discover novel therapeutic interventions to curtail the disease. One of the novel targets to control T2DM could be angiotensin-converting enzyme type 2 (ACE2), a member of the renin–angiotensin system (RAS) (1–4). The system has been extensively studied in the context of blood pressure regulation; however, we are just beginning to understand the role of the RAS in glucose homeostasis.

Modulation of the RAS Impacts Glucose Homeostasis

Increased RAS overactivity has been reported to contribute toward impaired glycemia (5–9). On the other hand, blockade of the RAS has been shown to be beneficial in improving glycemia in clinical studies (9–11). Components of the RAS (Fig. 1) have been identified not only in systemic circulation but also in
different tissues such as the eye (12), brain (13), heart (14), blood vessels (15–17), pancreas (18) and the islet of Langerhans (19–21), suggesting the existence of paracrine, intracrine and endocrine RAS. Angiotensin-II (Ang-II), one of the most important members of the RAS, is a potent vasoconstrictor that is involved in mediating disorders such as hypertension, heart failure, stroke and metabolic syndrome. Ang-II impairs glucose tolerance by inducing insulin resistance and blunting insulin secretion from the islets in face of hyperglycemia (22–24).

Ang-II has been reported to be associated with body fat accumulation and insulin resistance in obese patients with diabetes (6). Indeed, Ang-II not only affects insulin secretion from the islets but it also adversely affects insulin signaling by stimulating multiple serine phosphorylation of the insulin receptor β-subunit IRS-1, and the p85 regulatory subunit of PI3-kinase in the insulin signaling cascade (22,23). Interestingly, Ang-II-mediated detrimental effects on insulin signaling can be reversed by angiotensin II type 1 receptor blockers (ARBs). For example, irbesartan improved insulin sensitivity and glucose tolerance in the skeletal muscle of insulin-resistant obese Zucker rats by increasing the GLUT4 protein, insulin-dependent glucose transporter, expression (25). Moreover, valsartan has been demonstrated to improve glycemia by enhancing insulin signaling pathway in T2DM KK-Ay mice (26). Altogether, there is enough evidence to claim that the RAS plays a significant role in controlling glycemia and therefore this system can be targeted to develop novel therapies for T2DM.

ACE2: An Endogenous Protective Agent Against Overactive RAS

ACE2, a homologue of ACE (27,28), is highly expressed in kidney, heart, lung (29) and testis (27,28). Interestingly, it has also been reported to be localized in glucose-regulating tissues such as pancreas, including β-cells (3,30–32), adipose tissue (29) and liver (33). ACE2 belongs to the M2 zinc metalloproteinase family and is 42% identical in sequence with human somatic ACE in the regions surrounding the catalytic active sites. ACE2 has only a single catalytic domain unlike somatic ACE which has two active sites (N- and C-domains). ACE2 is a carboxypeptidase that cleaves a single basic residue from the C-terminus of its target peptides such as Ang-II, Ang-I, dynorphin (1–13), des-Arg5-bradykinin, apelin 13 and apelin 36 (34).

ACE2 degrades Ang-I and Ang-II to produce Ang-(1–9) and Ang-(1–7), respectively; however, the catalytic efficiency for Ang-II is much higher than for Ang-I (34), suggesting that the conversion of Ang-II to Ang-(1–7) is a physiologically more important reaction in vivo. Ang-(1–7), an endogenous ligand for the Mas receptor (MasR), is a vasodilator peptide that opposes some of the effects of the vasoconstrictor Ang-II (35,36). Hence, ACE2 seems to be a protective agent against the detrimental effects mediated through an overactive RAS by decreasing the levels of Ang-II and increasing the levels of Ang-(1–7). This therapeutic property of ACE2 is gaining a lot of attention, given the ubiquitous role of the RAS in regulating various functions in the body.
ACE2 plays a beneficial role in attenuating hypertension (37–40). In several animal models of hypertension, the levels of kidney and brain ACE2 protein expression have been found to be decreased compared to control groups. For example, spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SHRSP) manifest significantly lower kidney (41) and brain (42) ACE2 expression compared to control rats. It is opined that deficiency of ACE2, in those hypertensive animals, results in a build-up of excess Ang-II and a decrease in Ang-(1–7), predisposing animals to several disorders.

Interestingly, ACE2 also plays a major role in glucose homeostasis. Our laboratory recently reported that ACE2 knockout (KO) mice have significantly higher fasting blood glucose levels than age-matched wild-type (WT) littermates (4). Moreover, Niu et al. (3) demonstrated that ACE2 KO mice exhibit impaired glucose tolerance at different ages compared to control WT mice. The ACE2 KO mice display a decrease in first-phase insulin secretion in response to a glucose challenge, implying that ACE2 plays a role in the regulation of insulin secretion (3). On the other hand, overexpression of ACE2 improves islet function and glycemic control in diabetic mice (1). Adenoviral delivery of the human ACE2 gene to the pancreas of mice causes pancreatic and hepatic ACE2 overexpression and subsequent decrease of fasting blood glucose levels in diabetic db/db mice (1). ACE2 further improves glucose tolerance, increases islet insulin content, stimulates proliferation of pancreatic β-cells and reduces apoptosis in islets at the early stage of diabetes (8-week-old db/db mice), compared to control mice. In addition, Takeda et al. (2) recently reported the significance of ACE2 in improving glycemia. The study reported that deficiency of ACE2 exacerbates high-fat diet-mediated impaired glycemia in mice by reducing the expression of GLUT4 and myocyte enhancer factor 2A in the skeletal muscle. Moreover, pancreatic ACE2 counteracts Ang-II-mediated impaired glucose tolerance and insulin resistance (43) in mice. Beyond glycemia, ACE2 has also been reported to exhibit beneficial effects in ameliorating the complications of type 2 diabetes (44,45). In summary, all the reported preclinical studies regarding ACE2 and T2DM consistently corroborate the hypothesis that endogenous ACE2 is an important regulator of glycemia.

### Potential Mechanisms of Action of ACE2 in Regulating Glycemia

We propose that ACE2 acts by possibly attenuating the deleterious effects of Ang-II on vasoconstriction, fibrosis, inflammation, endoplasmic reticulum (ER) stress and β-cell death in the pancreas, thereby protecting a critical β-cell mass essential for insulin production (1,4) (Fig. 2). Our group recently showed for the first time the direct effect of ACE2 on insulin secretion in the mouse islets cultured in the presence of Ang-II (43) Ang-II disrupts β-cell function and thus prevents the increase in insulin release from the islets in the presence of high glucose concentration. On the other hand, this detrimental effect of Ang-II is attenuated by ACE2 gene therapy which normalizes insulin secretion from the islets when exposed to high glucose levels. The therapeutic effect of ACE2 seems to be mediated by negatively regulating Ang-II signaling in the β-cell. Indeed, we reported the blunting of Ang-II-mediated angiotensin II type 1 receptor (AT1R) upregulation in the islets of mice treated with an ACE2 adenovirus (43). Therefore, ACE2 has a potential to ameliorate AT1R-mediated detrimental consequences such as oxidative stress, blood flow, inflammation and fibrosis in the β-cell.

Several groups, including ours, previously reported that ACE2 can reduce Ang-II-mediated oxidative stress (43,45–47). Ang-II increases superoxide radicals and oxidative stress by inducing NADPH oxidase production (48) which in turn results in dysfunction of β-cells (49). We found that ACE2 treatment dramatically attenuated pancreatic oxidative stress and improved glycemia in Ang-II-infused mice. In addition, we showed that an antioxidant tempol improved insulin secretion in the presence of Ang-II in the mouse islets. Conversely, it has been reported that ACE2 inhibition or MasR blockade exacerbate Ang-II-mediated increase in reactive oxygen species within renal cell nuclei from sheep (50). The ACE2/Ang-(1–7)/MasR pathway thus appears to protect cells against oxidative stress (50). Therefore, reduction of oxidative stress might be a mechanism by which ACE2 can ameliorate the symptoms of T2DM as oxidative stress is reported to promote this disease (51). Altogether, studies suggest that ACE2 can improve β-cell function and glycemia by attenuating Ang-II-mediated oxidative stress.

Reports by Carlsson et al. (8,52) suggested that modulation of blood flow to the islets impacts whole-body glucose homeostasis. Ang-II, by virtue of its vasoconstrictive property, reduces the blood flow to the islets and negatively affects insulin secretion (8,43). To our surprise, ACE2 therapy did not improve blood flow to the pancreas in Ang-II-infused mice in our study (43). These conflicting data suggest that the role of ACE2 on blood flow to the islets needs to be further confirmed with different doses of ACE2 in animal models of RAS overactivity.

Islet inflammation and fibrosis significantly contribute toward β-cell dysfunction (53,54), thereby impacting glycemia. Agents that decrease islet inflammation and fibrosis have been shown to improve glycemia in diabetic subjects (54). Although the beneficial actions of ACE2 in attenuating fibrosis and inflammation have been reported in heart (55,56), lungs (39) and liver (57), the role of ACE2 therapy in possibly ameliorating those detrimental processes in the islets remain to be investigated. Recently, our study showed that ACE2 therapy attenuates pancreatic fibrosis, as suggested by the attenuation of collagen gene expression, in HFD-fed mice (58). Indeed, it is tempting to speculate the potential of ACE2 in abating islet inflammation and fibrosis, given the claim that Ang-II mediates both inflammation and fibrosis through AT1R in the islets (30,59,60).

ER stress is a novel emerging mechanism that has been shown to contribute to chronic diseases such as hypertension.
and diabetes (61,62). Interestingly, RAS overactivity has been reported to induce ER stress (61); conversely, drugs that can reduce RAS activity might attenuate ER stress in hypertension. ER stress appears to be one of the responsible factors for β-cell dysfunction and impaired glycemia (63); however, the role of Ang-II and ACE2 in mediating and attenuating ER stress, respectively, in the islets has not yet been determined.

ACE2 Shedding: A Link Between RAS Overactivity and Impaired Glycemia?

ACE2 expression and activity in various tissues decrease, as time progresses, in disorders such as T2DM and hypertension (1,43,64,65). The mechanisms for the observed reduction of ACE2 in those diseases remain unknown. One of the explanations for the diminishing ACE2 levels could be its shedding from the tissues (Fig. 3). A disintegrin and metalloproteinase (ADAM17) or TNF-α converting enzyme, a sheddase, has been reported to cleave cell-membrane-bound ACE2 (66) and thereby removes a part of the protein, containing the catalytic domain, from the tissues into the circulation. Indeed, ADAM17 is upregulated in human carotid atherosclerotic plaques of T2DM patients (67) and could be responsible for ACE2 shedding in this disease. This hypothesis is currently being tested in our laboratory.

Interestingly, Ang-II upregulates ADAM17 expression in vascular smooth muscle cells, the brain, the adipose tissues and the pancreas (68–72). ADAM17-induced ACE2 shedding provides an explanation for the observed downregulation of ACE2 in face of RAS overactivity in the mouse islets (71). The increase in ADAM17 expression is attenuated by ACE2 treatment, suggesting that modulation of Ang-II levels can impact ADAM17 levels (71). In summary, based on the preliminary studies, it would be logical to speculate that ACE2 shedding in the islets could be a possible link between RAS overactivity and impaired glycemia; restoration of shed ACE2 appears to control RAS activity and thus improves glycemia. Further studies should be aimed at investigating the interaction between ACE2 shedding and glycemia to confirm these preliminary findings.
Strategies to Restore Shed ACE2 in T2DM

In an effort to increase ACE2 activity in T2DM and other disorders, several approaches are under investigation at the preclinical stage. The most widely utilized strategy is the gene-therapy approach to increase ACE2 activity in animal models (1,37,40,47). Indeed, beneficial effects of ACE2 gene therapy have been successfully investigated in various disorders including T2DM by other groups (39,40) and our laboratory (1,4,38,47,73). The studies utilize either adenovirus or lentivirus, carrying the ACE2 gene, to increase ACE2 activity for a short (7–14 days) or long term, respectively. So far, the therapy has shown promising results in diabetic animals.

Activators of endogenous ACE2 such as xanthone and diminazene aceturate have been identified as compounds capable of increasing ACE2 activity to counteract the overactive RAS (74). ACE2 activators appear to be beneficial in attenuating hyperglycemia as well as diabetic complications such as hypertension and endothelial dysfunction in diabetic subjects (74–76). Thus, ACE2 activators provide a novel avenue to control T2DM and related complications.

Human recombinant ACE2 (hrACE2) has shown promising results in increasing ACE2 activity and controlling T2DM complications in animals (45). Recent reports suggest the role of hrACE2 therapy in opposing Ang-II-mediated signaling in diabetic nephropathy. Indeed, hrACE2 treatment attenuated diabetic kidney injury in the Akita mouse by reducing Ang-II-mediated NADPH oxidase activity and increasing Ang-(1–7) signaling (45). These encouraging results provide the rationale for determining the effects of rhACE2 on glycemia and insulin secretion from the β-cells, given the negative role of NADPH oxidase and Ang-II in T2DM. Therefore, future studies should be aimed at confirming the beneficial role of rhACE2 in improving glycemia in T2DM animal models.

Is ACE2 Therapy Superior to ARB and ACE Inhibitors for the Regulation of Glycemia?

ACE inhibitors do not completely block the formation of Ang-II as there are alternative enzymes such as chymase (77) which contribute to the formation of Ang-II and could mediate hyperglycemia. Although ARBs block AT1 receptor and prevent the detrimental effects of Ang-II, a recent study suggested that the beneficial effects of olmesartan, an ARB, on vascular remodeling are mediated via activation of ACE2-Ang(1–7)-Mas axis of RAS (78). This possible interplay between ARBs and ACE2-Ang(1–7)-Mas axis suggests the importance of ACE2 therapy over other RAS blockers in combating hyperglycemia induced by increased Ang-II/AT1R signaling. ACE2, on the other hand, acts directly on Ang-II, irrespective of the pathways involved in the production of this octapeptide (27). Thus, by ensuring depletion of Ang-II in the body, ACE2 therapy seems more efficient than ACE inhibitors—in the presence of which Ang-II can still be produced in the body—in combating Ang-II-mediated hyperglycemia. ARBs and ACE inhibitors improve glycemia through blocking RAS overactivity. In contrast, ACE2 not only counteracts RAS overactivity but also increases Ang-(1–7)/MasR signaling in different cell types. ACE2 converts Ang-II to Ang-(1–7), which exerts its effects by binding to the MasR. Ang-(1–7)-Mas signaling tends to rescue the increase in blood pressure mediated by Ang-II. Ang-(1–7) infusion alone, in the presence of RAS overactivity, has proven to be beneficial in improving glycemia (79). Ang-(1–7) has been reported to be beneficial in diabetic

FIG 3  ADAM17-mediated ACE2 shedding in β-cell of the pancreas. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
SHR by improving glycemia, renal vascular function, reducing NADPH-mediated oxidative stress and proteinuria (79,80). Like ACE2, infusion of Ang-(1–7) can improve insulin sensitivity (81) and lower blood pressure in a rat model of metabolic syndrome (82). Indeed, transgenic rats exhibiting elevated systemic Ang-(1–7) levels manifest improved glucose and lipid metabolism (83). Moreover, Ang-(1–7) infusion ameliorates the diabetic complications in diabetic rats (84). Interestingly, the beneficial effects conferred by ACE inhibition were associated with an increase in plasma levels of Ang-(1–7) in the SHR (85). Pregnancy tends to elevate the plasma level of Ang-(1–7), but women with gestational diabetes have lower levels of Ang-(1–7) than healthy pregnant women, suggesting a role for Ang-(1–7) in the maintenance of glycemia during pregnancy (86). More importantly, blockade of the Ang-(1–7) receptor with the D-Ala7-Ang-(1–7) antagonist, opposes the effects of ACE2 over-expressing (1), which emphasizes the role of Ang-(1–7) in β-cell function and glycemia.

The importance of the MasR can be illustrated by a study showing that Mas KO mice in the FVB/N background manifest high blood pressure, low levels of nitric oxide (NO) and a low concentration of the mRNA for endothelial NO synthase (an enzyme that synthesizes NO in the blood vessel wall) (87). Genetic deletion of the MasR in the FVB/N background also leads to a metabolic syndrome-like state in the form of dyslipidemia, hyperinsulinemia, impaired glucose tolerance and diminished insulin sensitivity (88). These effects could be related to our recent observation that MasR is located on β-cells (50). Moreover, AVE0991, a MasR agonist, plays a cardioprotective role in diabetic rats (89) and has also been reported to ameliorate the extent of diabetic cardiovascular dysfunction in rats with streptozotocin-induced diabetes (84).

In summary, unlike ARBs and ACE2 inhibitors, ACE2 mediates its favorable actions by modulating two pathways: 1) degrading detrimental Ang-II levels and controlling RAS activity; 2) increasing Ang-(1–7), which, through its specific G-protein-coupled receptor Mas, mediates beneficial effects such as improved insulin sensitivity, reduced oxidative stress and vasodilation that are of enormous significance in diabetes.

Perspective

T2DM is growing into an epidemic proportion worldwide. Preclinical studies reveal that ACE2 seems to be a promising therapeutic agent to control T2DM. The classic ACE/Ang-II/AT1R axis of the RAS is one of the contributing factors that mediates diabetes, whereas the compensatory ACE2/Ang-(1–7)/MasR arm provides a novel approach to improve glycemia in T2DM (Fig. 4).

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