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Review

The sweeter side of ACE2: Physiological evidence for a role in diabetes

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ABSTRACT

Diabetes mellitus is a growing problem in all parts of the world. Both clinical trials and animal models of type I and type II diabetes have shown that hyperactivity of angiotensin-II (Ang-II) signaling pathways contribute to the development of diabetes and diabetic complications. Of clinical relevance, blockade of the renin–angiotensin system prevents new-onset diabetes and reduces the risk of diabetic complications. Angiotensin-converting enzyme (ACE) 2 is a recently discovered mono-carboxypeptidase and the first homolog of ACE. It is thought to inhibit Ang-II signaling cascades mostly by cleaving Ang-II to generate Ang-(1–7), which effects oppose Ang-II and are mediated by the Mas receptor. The enzyme is present in the kidney, liver, adipose tissue and pancreas. Its expression is elevated in the endocrine pancreas in diabetes and in the early phase during diabetic nephropathy. ACE2 is hypothesized to act in a compensatory manner in both diabetes and diabetic nephropathy. Recently, we have shown the presence of the Mas receptor in the mouse pancreas and observed a reduction in Mas receptor immunoreactivity as well as higher fasting blood glucose levels in ACE2 knockout mice, indicating that these mice may be a new model to study the role of ACE2 in diabetes. In this review we will examine the role of the renin–angiotensin system in the physiopathology and treatment of diabetes and highlight the potential benefits of the ACE2/Ang-(1–7)/Mas receptor axis, focusing on recent data about ACE2.

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1. Introduction

Diabetes mellitus is characterized by insulin insufficiency due to either decreased insulin release or end-organ insulin resistance. Several major categories of diabetes exist, type I (T1DM) and type II (T2DM) diabetes, gestational diabetes and maturity onset diabetes of the young (MODY). T1DM usually occurs in children and young adults and is characterized by pancreatic β -cell failure or auto-immune destruction of the pancreatic β -cells. T2DM is characterized by insulin resistance and relative insulin insufficiency with adult onset. Risk factors for T2DM include family history, hypertension, obesity and dyslipidemia. MODY is characterized by specific defects in glucose metabolism.

Diabetes currently affects more than 135 million people worldwide. The number of diabetics is expected to grow to 300 million by the year 2025, which will represent approximately a 42 and 170% increases in the prevalence of diabetes in developed and developing countries, respectively (King et al., 1998). In addition to a rise in diabetes among adults, childhood T2DM is also expected to rise due to increasing childhood obesity (Dabelea et al., 1999; Pinhas-Hamiel and Zeitler, 2005). Although until recently non-immune-mediated diabetes was rare in children, the American Diabetes Association recently reported a rise in the number of children with new diagnoses of such disease, the majority of whom have T2DM (Anonymous, 2000b). Indeed, a rise in childhood T2DM has been observed across many ethnic groups including Native Americans (Dabelea et al., 1998), Hispanics (Neufeld et al., 1998), African-Americans (Dabelea et al., 1999) and Caucasians (Drake et al., 2002; Ehtisham et al., 2000).

Diabetes is a leading cause of morbidity and mortality, and diabetics are at increased risk for development of hypertension, myocardial infarction, renal disease and stroke (Grundey et al., 1999). Thus, the global increase in diabetes is a public health crisis which requires new prevention and treatment tools.

2. Tissue renin–angiotensin system (RAS) and metabolism

The classical renin–angiotensin system consists of liver-generated angiotensinogen being cleaved by renin, generated in the kidney, to produce angiotensin-I (Ang-I). Ang-I is subsequently

hydrolyzed by angiotensin-converting enzyme (ACE) to produce angiotensin-II (Ang-II), which promotes vasoconstriction, inflammation, salt and water reabsorption and oxidative stress. Two decades ago, the concept of local tissue RAS was introduced following the observation that many tissues are capable of synthesizing a majority of the RAS components locally (Dzau and Re, 1994; Lavoie and Sigmund, 2003).

2.1. Effect of the RAS on metabolic organs

The existence of a local RAS has been confirmed in many tissues, including the heart (Danser and Schalekamp, 1996), vasculature (Paul et al., 2006), brain (Baltatu et al., 2000), retina (Tikellis et al., 2000a) liver (Bataller et al., 2003; Paizis et al., 2002) and pancreas (Lau et al., 2004). The local RAS influences the function of the metabolic organs and the development of diabetes (Fig. 1). Expression of ACE, the Ang-II type 1 (AT1) receptor and renin have been identified in the liver by quantitative RT-PCR (Paizis et al., 2002) and protein expression confirmed by immunohistochemistry (Paizis et al., 2002). Ang-II type 2 (AT2) receptor expression has been reported in stellate cells (Bataller et al., 2003), although this could not be confirmed by other groups (Paizis et al., 2002). Studies involving RAS blockade in the setting of diabetes have given some clues to the potential role of the intra-hepatic RAS in the pathogenesis of diabetes. RAS blockade prevents hepatic triglyceride accumulation in obese Zucker rats, a common feature of insulin resistant and diabetic states (Toblli et al., 2007). Additionally, enalapril prevents fibrosis and oxidative stress in the liver, heart and kidney of rats with T1DM (de Cavanagh et al., 2001). The most remarkable evidence for the role of the RAS in diabetes is the demonstration that captopril suppressed hepatic glucose output and increased hepatic glucose uptake in response to insulin infusion (Torlone et al., 1991).

RAS components have been identified in both the endocrine and exocrine pancreas. Angiotensinogen, renin, AT1 and AT2 receptors, ACE and ACE2 proteins are all expressed in this organ (Tikellis et al., 2004b; Leung et al., 1999; Chappell et al., 1991; Carlsson, 2001; Leung et al., 1998). Additionally, AT1 receptor protein expression is limited to the pancreatic β -cell (Tahmasebi et al., 1999). While pro-renin mRNA expression was identified by in situ hybridization in the reticular fibers of the islets and perivascular connective

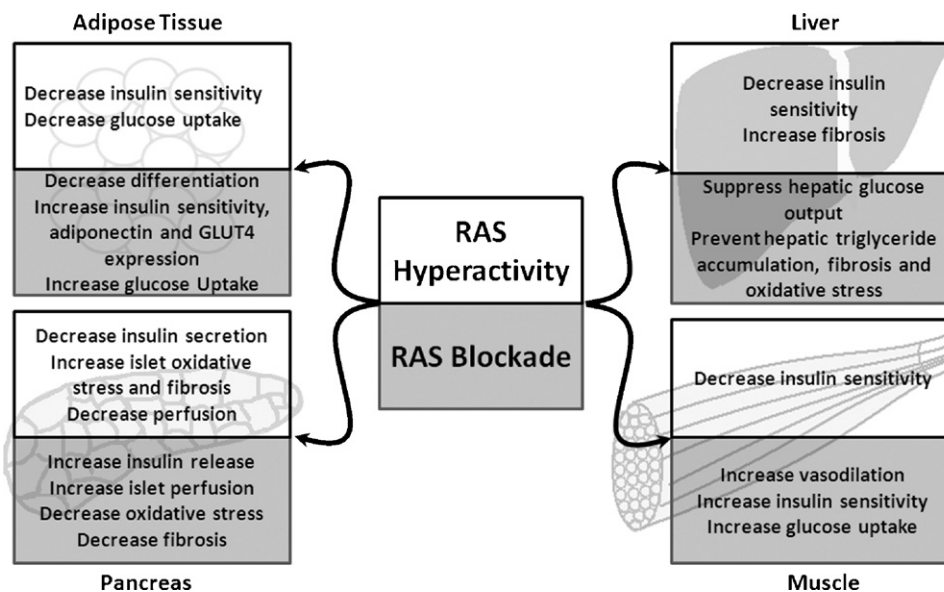


Fig. 1. The role of the RAS in metabolic organ function. Ang-II signaling cascades inhibit insulin release and decrease insulin sensitivity. RAS blockade improves glucose homeostasis by reducing β -cell death and improving insulin secretion and end-organ insulin sensitivity.

tissue, immuno-staining revealed that islet pro-renin protein primarily exists in the β -cells (Tahmasebi et al., 1999). Several studies have demonstrated activation of the islet RAS in diabetic animals (Leung, 2007; Tikellis et al., 2004b).

Maintenance of islet perfusion is important in the regulation of glucose-stimulated insulin secretion (GSIS) (Tikellis et al., 2006) and a potential role for the pancreas RAS has been suggested in the maintenance of islet blood flow (Leung, 2007). Supporting evidence include: (1) greater Ang-II levels in islet microvessels compared to exocrine pancreas microvessels (Carlsson et al., 1998); (2) acute Ang-II infusion decreases islet blood flow in healthy non-diabetic Sprague–Dawley rats (Carlsson et al., 1998); and (3) RAS blockade by ACE inhibitors or AT1 receptor blockers preferentially increased blood flow to islet microvessels (Carlsson et al., 1998).

Another role of the islet RAS is in the modulation of β -cell function. Insulin release occurs in two phases, the first phase appearing within 10–15 min after stimulus and peaking at 3 min (Curry et al., 1968). In non-diabetic rats, acute Ang-II infusion prevented the first phase insulin secretion in response to glucose (Carlsson et al., 1998), and in healthy human subjects, pressor doses of Ang-II suppressed basal, pulsatile and glucose-stimulated insulin release (Fliser et al., 1997). This loss of first phase insulin secretion is hypothesized to be the earliest detectable defect in the development of T2DM (Gerich, 2002).

2.2. Pancreatic RAS and oxidative stress

Although RAS inhibition has no effect on basal insulin secretion (Carlsson et al., 1998), it may improve insulin synthesis and secretion by protecting β -cells from oxidative stress-related tissue damage and loss of insulin secretion. Pancreatic islets express lower levels of antioxidants than the majority of other tissues in the body (Grankvist et al., 1981), which makes them especially vulnerable to oxidative stress (Evans et al., 2002). Several studies indicate that hyperglycemia produces reactive oxygen species (ROS) in islets and pancreatic β -cells (Wu et al., 2004; Tsubouchi et al., 2004; Tang et al., 2007; Bindokas et al., 2003), as illustrated in the diabetic Zucker rat (Tang et al., 2007). Moreover, oxidative stress induces pancreatic β -cell dysfunction (Robertson, 2004; Matsuoka et al., 1997; Maechler et al., 1999; Kaneto et al., 2001; Harmon et al., 2005). NAD(P)H oxidase (NOX) is one suggested source of ROS in the islet (Nakayama et al., 2005; Oliveira et al., 2003; Tsubouchi et al., 2004). NOX activation is triggered by interaction of Ang-II with the AT1 receptor (Griendling et al., 1994) and requires the assembly of the cytosolic subunits p47phox, p40phox and p67phox on the membrane bound scaffold proteins p22phox and Nox2/4 (Harrison et al., 2007). Expression of Nox2 (Oliveira et al., 2003), p22phox (Oliveira et al., 2003), and Nox4 (Uchizono et al., 2006) has been shown in pancreatic β -cells and is further increased in models of T1DM and T2DM (Nakayama et al., 2005; Oliveira et al., 2003; Tsubouchi et al., 2004).

In addition to causing pancreatic β -cell dysfunction and apoptosis, oxidative stress can lead to loss of insulin synthesis and secretion through several mechanisms including loss of transcription factors (e.g. MaFA and Pdx-1) required for insulin gene expression (Matsuoka et al., 1997; Kaneto et al., 2001). Secondly, ROS are implicated in the development of amyloid deposition (Hayden and Tyagi, 2002) and islet fibrosis (Hayden et al., 2007). Both have been linked to the development of β -cell dysfunction and apoptosis (Clark and Nilsson, 2004; Hayden et al., 2007). In agreement with the hypothesis that RAS activation leads to increased islet ROS and fibrosis, Hayden et al. demonstrated that Ren2 rats, with elevated Ang-II accumulation due to renin over-expression (Langheinrich et al., 1996), have elevated islet nitrotyrosine content and fibrosis (Hayden et al., 2007). In addition, islet nitrotyrosine

and TGF- β expression co-localize with areas of increased peri-islet fibrosis (Hayden et al., 2007). Enhanced fibrosis of islets and decreased insulin synthesis cumulatively can cause decreased insulin secretion and exacerbate the development of diabetes. RAS blockade has been shown to be effective at reducing oxidative stress-induced changes in islets by inhibiting the expression of the NOX components, p22phox and Nox2 (Nakayama et al., 2005), thus decreasing the expression of oxidative stress markers, including nitrotyrosine (Lupi et al., 2006; Tikellis et al., 2004a,b) and 4-hydroxy-2-nonenal modified protein, a marker of fatty acid oxidation (Nakayama et al., 2005). In addition, RAS blockade has been associated with a decrease in islet AT1 and ACE expression (Tikellis et al., 2004a,b), which would result in decreased NOX activation. Moreover, RAS blockade-mediated decreases in the expression of the NOX components p22phox and Nox2 (Shao et al., 2006), and nitrotyrosine levels (Shao et al., 2006; Tikellis et al., 2004b) in the islet were associated with prevention of islet dysfunction and deleterious changes in islet morphology. This was demonstrated by prevention of (1) islet fibrosis (Shao et al., 2006; Tikellis et al., 2004a,b), (2) loss of insulin secretion (Lupi et al., 2006; Shao et al., 2006; Tikellis et al., 2004b) and (3) loss of β -cells and reduction in islet insulin content (Shao et al., 2006; Tikellis et al., 2004b; Nakayama et al., 2005), independently of changes in plasma glucose levels. Therefore, hyperactivity of Ang-II signaling cascades is clearly implicated in the development of islet oxidative stress and subsequent β -cell dysfunction.

3. RAS blockade inhibits new onset diabetes: clinical trials evidence

Clinical trials have demonstrated the ability of RAS blockade to prevent new-onset diabetes and the development of diabetic complications. Meta-analysis of several trials demonstrated that ACE inhibitors or Ang-II receptor blockers (ARB) reduced the risk of diabetes by 25% (Abuissa et al., 2005). Interestingly, when compared to diuretic and β -blocker therapies, patients receiving ARB or ACE inhibitors had a 25–30% lower risk of developing new-onset diabetes despite similar reductions in blood pressure (BP) (Anonymous, 2000a; Niklason et al., 2004; Reid et al., 2003; Vermes et al., 2003). Indeed, β -blocker therapy has been shown to increase the risk of developing diabetes (Mason et al., 2005; Gress et al., 2000). These findings indicate that a reduction in BP alone is not sufficient to prevent new onset diabetes and that these effects are likely specific to RAS inhibition. In addition to the prevention of new-onset diabetes, RAS blockade ameliorates glycemic control in diabetic patients. It reduced glycosylated hemoglobin (HbA_{1c}) levels, a measure of long-term glycemic control (Anonymous, 1998; Yusuf et al., 2001), and reduced the use of oral anti-hyperglycemic agents in diabetics when compared to placebo or conventional therapy (Anonymous, 1998; Yusuf et al., 2001). The ability of RAS blockade to prevent new-onset diabetes and ameliorate glycemic control in diabetic patients combined with its ability to improve islet morphology, insulin secretion and insulin sensitivity provide powerful evidence that the RAS should be a continued target for novel therapeutics in diabetes.

4. ACE2, a new member of the RAS

ACE2 was initially identified from human heart failure and lymphoma cDNA libraries (Donoghue et al., 2000; Tipnis et al., 2000) and was later shown to serve as a receptor for the SARS coronavirus (Li et al., 2003). ACE2 is the first ACE homologue with 42% sequence homology, and unlike ACE, this zinc metalloprotease contains only one HEXXH consensus sequence, resulting in

monocarboxy-peptidase activity (Tipnis et al., 2000). In addition, ACE2 also has 48% sequence homology with collectrin, a newly discovered glycoprotein responsible for regulation of renal amino acid transport (Danilczyk et al., 2006; Zhang et al., 2001) and maintenance of collecting duct cell morphology (Zhang et al., 2007). Both proteins share identity in the cytosolic, sheddase, transmembrane and non-catalytic extracellular domains (Zhang et al., 2001). ACE2 exists as both membrane bound and soluble forms (Donoghue et al., 2000; Feng et al., 2008), the latter being generated by proteolytic cleavage of the ectodomain by the tumor necrosis factor convertase, ADAM17 (Lambert et al., 2005). Although ACE2 shares the catalytic domain of ACE, it is not sensitive to ACE inhibitors (Tipnis et al., 2000).

ACE2 substrate specificity has been hypothesized to require a Pro-X-Pro-hydrophobic/basic conformation for proteolytic cleavage (Guy et al., 2003). While the carboxypeptidase is capable of cleaving the terminal leucine from Ang-I to generate Ang-(1–9) (Donoghue et al., 2000), it has approximately a 400-fold greater affinity for Ang-II (Vickers et al., 2002). ACE2 cleaves the terminal phenylalanine residue from Ang-II to generate Ang-(1–7) (Tipnis et al., 2000). Although the enzyme is also capable of complete hydrolysis of des-Arg⁹-bradykinin, apelin-13, β -casomorphin, neocasinomorphin and dynorphin-A, and partial hydrolysis of many biologically active peptides including neurotensin and ghrelin (Vickers et al., 2002), only its effects on angiotensin peptides have been investigated so far.

Since its original detection primarily in human heart, kidneys and testes (Donoghue et al., 2000), ACE2 has been identified in a variety of tissues, including the rat brain (Gallagher et al., 2003), lung (Gembardt et al., 2005), adipose tissue (Gembardt et al., 2005), liver (Paizis et al., 2005), pancreas (Tikellis et al., 2004a) retina (Tikellis et al., 2004b), and human placenta (Valdés et al., 2006), colon, small intestine, ovary (Tipnis et al., 2000), and is thought to be ubiquitously expressed. It has been hypothesized to regulate the RAS by opposing the ACE/Ang-II/AT1 receptor axis, and therefore counter several pathologic processes including cardiac dysfunction (Diez-Freire et al., 2006; Gurley et al., 2006; Yamamoto et al., 2006), hypertension (Diez-Freire et al., 2006; Gurley et al., 2006; Yamazato et al., 2007), acute respiratory distress syndrome (Imai et al., 2008, 2005), inflammation (Huentelman et al., 2005; Paizis et al., 2005; Diez-Freire et al., 2006) and fibrosis (Diez-Freire et al., 2006; Huentelman et al., 2005; Paizis et al., 2005; Herath et al., 2007). While Ang-II has well known vasoconstrictor, pro-inflammatory and pro-oxidant effects, mediated mostly by the AT1 receptor, Ang-(1–7), the primary product of Ang-II degradation by ACE2, acts through the Mas receptor (Santos et al., 2003) to produce vasodilation via activation of bradykinin and nitric oxide (NO) (Oliveira et al., 1999; Brosnihan et al., 1996; Fernandes et al., 2001; Paula et al., 1995; Toblli et al., 2007), release of prostaglandins (Almeida et al., 2000), and inhibition of norepinephrine release (Gironacci et al., 2004). Recent studies have implicated the Akt pathway in Ang-(1–7) mediated NO release (Sampaio et al., 2007). In addition, ACE2 attenuates fibrosis and inflammation through inhibition of TGF- β (Grobe et al., 2007) and macrophage migration inhibitory factor (MIF) (Zhong et al., 2007).

5. ACE2 and diabetes

The study of ACE2 in the context of diabetes has been essentially focused on the kidney. ACE2 may be an important target for the treatment and prevention of diabetic nephropathy, a progressive condition characterized by mesangial matrix expansion, thickening of the glomerular basement membrane, proteinuria, and associated with chronic renal failure. Recently several studies have also implicated podocyte loss (Schmid et al., 2003; Susztak et al., 2006; Dalla

Vestra et al., 2003; Meyer et al., 1999; Pagtalunan et al., 1997) and this disappearance is associated with worsening glomerular injury (Yu et al., 2005).

The kidney expresses a fully functional local RAS, capable of producing Ang-II (Bader et al., 2001; Paul et al., 2006), which is activated in hyperglycemic conditions (Konoshita et al., 2006). ACE2 expression has been identified in renal cortical tubules (Ye et al., 2004) and apical border of the proximal convoluted tubules (Ye et al., 2006) in mice, where ACE2 co-localizes with podocyte specific markers (Schmid et al., 2003) nephrin, podocin and synaptopodin. Electron microscopy further confirmed that ACE2 was primarily located in the podocyte (Ye et al., 2006).

ACE2 expression in the kidney has been studied in both T1DM and T2DM models. In 8-week-old db/db mice, a model of early T2DM, ACE2 expression is elevated while ACE expression is decreased in both glomeruli and cortex (Ye et al., 2004) prior to the development of diabetic nephropathy. Similarly, in streptozotocin-induced diabetic mice, a T1DM model, ACE2 protein expression is elevated in the early (Wysocki et al., 2006) and decreased in the late (Tikellis et al., 2003) phase of diabetic nephropathy. Additionally, in humans, ACE expression is increased while ACE2 expression is decreased in the tubules in diabetic nephropathy (Mizuiiri et al., 2008). Decreased ACE2 expression was associated with increased albuminuria (Tikellis et al., 2003) and pharmacological inhibition of ACE2 by MLN-4760 increased urinary albumin excretion 3–4-fold in both T1DM and T2DM models (Soler et al., 2007; Ye et al., 2006). MLN-4760 treatment exacerbated mesangial matrix expansion, vascular thickness (Soler et al., 2007) and also caused focal loss of podocytes in streptozotocin-induced diabetic mice, indicating that ACE2 may be required for podocyte maintenance. Finally in two models of T1DM (streptozotocin and Akita mouse models), ACE2 gene deletion accelerated the development of diabetic kidney disease (Wong et al., 2007; Tikellis et al., 2008). Akita mice are a genetic model of T1DM with reduced β -cell activity but without evidence of insulinitis (Yoshioka et al., 1997). Interestingly, ACE2 gene deletion in a Akita mouse background, by crossing Akita with ACE2 knockout (ACE2^{-/-}) mice, resulted in increased albuminuria as well as fibronectin and smooth muscle actin expression compared to diabetic mice with intact ACE2 (Wong et al., 2007). On the other hand, in streptozotocin-induced diabetic ACE2^{-/-} mice, ACE2 gene deletion inhibited the beneficial effects of ACE inhibitor (Tikellis et al., 2008) in diabetic nephropathy, suggesting that ACE inhibition potentiates ACE2 activity. ARB therapy did however reduce albuminuria in Akita-ACE2^{-/-} mice (Wong et al., 2007). Interestingly, Ang-(1–7) administration also worsened diabetic kidney injury in streptozotocin-induced diabetes, indicating that the effects of ACE2 may be mediated by the loss of Ang-II rather than via Ang-(1–7) (Shao et al., 2008). The current findings suggest that ACE2 may participate in a compensatory mechanism in the diabetic kidney prior to the onset of diabetic nephropathy. Indeed, several studies demonstrated that loss or inhibition of ACE2 exacerbates diabetic nephropathy (Ye et al., 2006; Soler et al., 2007; Wong et al., 2007; Tikellis et al., 2008). Moreover, the localization of ACE2 in the podocytes early in the development of diabetes indicates that it may protect against podocyte loss, thus preventing the worsening glomerular injury (Yu et al., 2005) and confirming ACE2 as an important target for future studies of diabetic kidney disease.

6. ACE2 in the endocrine pancreas

Expression of both ACE2 (Tikellis et al., 2004b) and Ang-(1–7) (Chappell et al., 1991) has been identified in the rat and canine endocrine pancreas. In diabetic Zucker fatty rats, a genetic model of T2DM characterized by obesity, hyperglycemia, reduced insulin sensitivity and secretion (Clark et al., 1983), ACE2 protein and mRNA

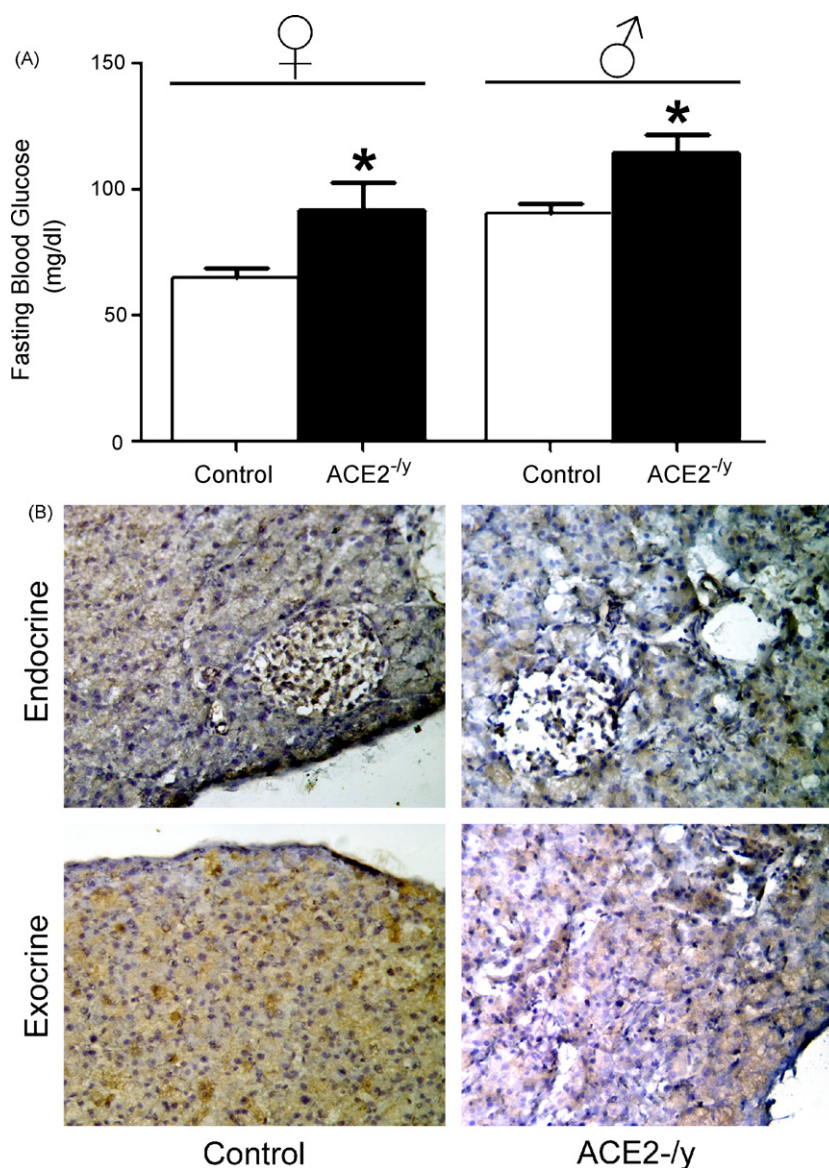


Fig. 2. Consequences of ACE2 gene deletion. (A) Following a 12-h fast, fasting blood glucose levels were measured in females ($n=5$) and males ($n=8$) ACE2 knockout mice using an Accu-Check[®] Aviva glucometer (Roche). ACE2 knockout mice have elevated fasting blood glucose in comparison to age-matched littermates. Data are expressed as mean \pm S.E.M. * $P < 0.05$; statistical significance vs. control mice. (B) Mas receptor immunohistochemistry reveals expression of this Ang-(1–7) receptor in the mouse endocrine and exocrine pancreas. Mas receptor expression was reduced in ACE2^{-/-} mice. Pancreas sections (16 μ m) were incubated with a rabbit anti-Mas antibody (AbCam) for 18 h at 4 °C and developed using the standard ABC method (Vector Laboratories) using DAB as the chromagen. A brown staining is indicative of Mas receptor immuno-reactivity.

expression are elevated in both acini and islets and correlated to an increase in ACE, collagen IV and TGF- β 1 levels (Tikellis et al., 2004b). Although ACE2^{-/-} mice are not diabetic, we observed a significant increase in fasting blood glucose in both males (90 ± 4 vs. 115 ± 7 ; $P < 0.05$) and females (65 ± 4 vs. 92 ± 10 ; $P < 0.05$) ACE2^{-/-} mice compared to littermates (Fig. 2A). However, there was no significant difference in body weight in comparison to littermates (23.3 ± 1.2 vs. 23.1 ± 0.9 , $P > 0.05$). We also observed Mas receptor expression in the pancreas, which was decreased in ACE2^{-/-} mice (Fig. 2B). ACE2 and therefore Ang-(1–7) may potentially influence islet function through modulation of blood flow and inhibition of fibrosis, via modulation of NO release. Islet blood flow is enhanced during increased functional demand due to glucose infusion (Styrud et al., 1992), obesity (Atef et al., 1992), pancreatectomy (Jansson and Sandler, 1981) and diabetes (Carlsson et al., 1996, 1997). Notably, enhanced islet perfusion is accompanied by an increase in islet capillary BP in Goto-Kakizaki rat, a model of T2DM (Carlsson et al.,

1997). Altered islet blood flow has been associated with abnormal secretion of glucagon (Samols and Stagner, 1988) and insulin (Atef et al., 1994). NO regulates both basal (Carlsson et al., 1996) and glucose-mediated islet blood flow (Moldovan et al., 1996). Elevated ACE2 levels in diabetes may lead to increased Ang-(1–7) levels in the islet, which could result in vasodilation through stimulation of NO release (Portsi et al., 1994; Heitsch et al., 2001). Accordingly, increased Ang-(1–7) stimulation of NO signaling would then be a potential mechanism for increased islet blood flow seen in T1DM and T2DM.

Ang-II is well known for its pro-inflammatory (Suzuki et al., 2003) and pro-fibrotic actions (Mezzano et al., 2001; Weber et al., 1993), while several studies have demonstrated the ability of ACE2 to inhibit these effects in disease states (Diez-Freire et al., 2006; Huentelman et al., 2005; Paizis et al., 2005; Herath et al., 2007). In addition, TGF- β plays an important role in the development of islet dysfunction and loss of islet morphology (Donath et al., 2003),

which is one potential cause of pancreatic β -cell failure (Johnson et al., 2003). TGF- β is implicated in the development of fibrosis in many disease states (Branton and Kopp, 1999) and its expression is elevated in the islets of mice with T2DM (Tikellis et al., 2004b; Yamamoto et al., 1993). On the other hand, RAS blockade decreases TGF- β expression in the islet and is associated with improved islet morphology and function (Tikellis et al., 2004b). While the effects of ACE2 on the preservation of islet architecture have not been investigated, combined evidence of the effectiveness of ACE2 at inhibiting fibrosis (Diez-Freire et al., 2006; Huentelman et al., 2005) and the ability of the enzyme to reduce TGF- β expression in models of cardiac fibrosis (Grobe et al., 2007) suggest that ACE2-mediated inhibition of TGF- β expression may prevent islet fibrosis and loss of islet function.

7. Potential role for ACE2 in insulin resistance

While there have been no studies on the effect of ACE2 or Ang-(1–7) on glucose metabolism in the liver, ACE2 has been identified in hepatocytes (Paizis et al., 2005). Along with other RAS components, ACE2 is elevated in hepatic fibrogenic diseases (Paizis et al., 2005; Herath et al., 2007). Mas receptor expression has also been identified in the liver and is elevated in biliary fibrosis (Herath et al., 2007). ACE2 expression is also elevated in hypoxic conditions in the liver (Paizis et al., 2005), supporting a potential compensatory role for the carboxypeptidase.

Although ACE2 mRNA has also been identified in white adipose tissue (Galvez-Prieto et al., 2008; Gembardt et al., 2005), the physiological implications of this observation have not been studied. Santos et al. however, recently reported the effects of Mas receptor deficiency on metabolism (Santos et al., 2007). Ang-(1–7) binding to the G-protein-coupled Mas receptor (Santos et al., 2003) has been shown to inhibit Ang-II responses (da Costa Goncalves et al., 2007; Tallant et al., 2005; Sampaio et al., 2007). Mas knockout (Mas^{-/-}) mice exhibit many common features of metabolic syndrome (Santos et al., 2007), an aforementioned risk factor for T2DM. While there was no difference in body weight or food intake, Mas^{-/-} mice have elevated leptin and insulin levels and increased epididymal and retroperitoneal fat mass. Additionally, these mice are dyslipidemic, exhibiting elevated serum and muscle triglycerides and blood cholesterol levels. Mas^{-/-} mice also exhibit impaired insulin sensitivity and glucose tolerance, reduced adipose glucose uptake and impaired glucose transporter 4 (GLUT4) expression (Santos et al., 2007). This study clearly demonstrates that loss of the Mas receptor leads to metabolic dysfunction and implicates the ACE2/Ang-(1–7)/Mas receptor axis in the development of insulin resistance.

Obesity and insulin resistance are associated with impaired endothelium-dependent and insulin-mediated vasodilation (Laakso et al., 1990; Steinberg et al., 1996; Williams et al., 1996). Moreover, obesity, insulin resistance, and diabetes are associated with decreased exercise-induced hyperemia (Fossum et al., 1998; Frisbee, 2006; Xiang et al., 2008), and exercise-induced muscle glucose uptake (Laine et al., 1998; Utriainen et al., 1998; Peltoniemi et al., 2001). In both T1DM and T2DM, glucose transport from the blood to the skeletal muscle has been shown to be the major impediment to skeletal muscle glucose uptake (Fueger et al., 2004; Baron et al., 1991). It is well known that Ang-(1–7) potentiates bradykinin-mediated vasodilation (Paula et al., 1995; Lima et al., 1997). In both T1DM and T2DM, loss of Ang-(1–7) potentiation of bradykinin-mediated vasodilation is rescued by chronic but not acute insulin (Oliveira et al., 2002; Rastelli et al., 2007). While Oliviera et al. demonstrated that Ang-(1–7) potentiation of bradykinin was not dependent on NO but instead on voltage gated K⁺ channels (Oliveira et al., 2002), Rastelli et al. showed that NO,

K⁺ channels and prostanoids influenced this pathway (Rastelli et al., 2007).

In addition to modulation of vasodilation, bradykinin signaling increases GLUT4 translocation (Kishi et al., 1998) and glucose uptake in skeletal muscle and adipose tissue (Beard et al., 2006; Kishi et al., 1998). Moreover, bradykinin-mediated enhancement of glucose uptake in the adipose tissue has been shown to involve NO (Beard et al., 2006). Indeed, ACE inhibitors improve skeletal muscle glucose uptake through a bradykinin-mediated increase in NO (Henriksen et al., 1999). Ang-(1–7)-mediated potentiation of bradykinin signaling may then also affect GLUT4 translocation and glucose uptake by the skeletal muscle. Blunting of this pathway may thus exacerbate the development of insulin resistance and diabetes.

8. Summary

Hyperactivity of the RAS is involved in the pathogenesis of diabetes and diabetic complications. ACE2, a new component of the RAS, is elevated in diabetes and diabetic nephropathy and may be involved in a compensatory mechanism opposing the ACE/Ang-II/AT1-receptor axis. In the islets, ACE2 may modulate blood flow and morphology in order to maintain insulin secretion (Fig. 3). This compensatory effect of ACE2 in diabetes has already been suggested in the kidney by studies demonstrating that ACE2 inhibition worsens diabetic nephropathy. In addition, Ang-(1–7), the product of

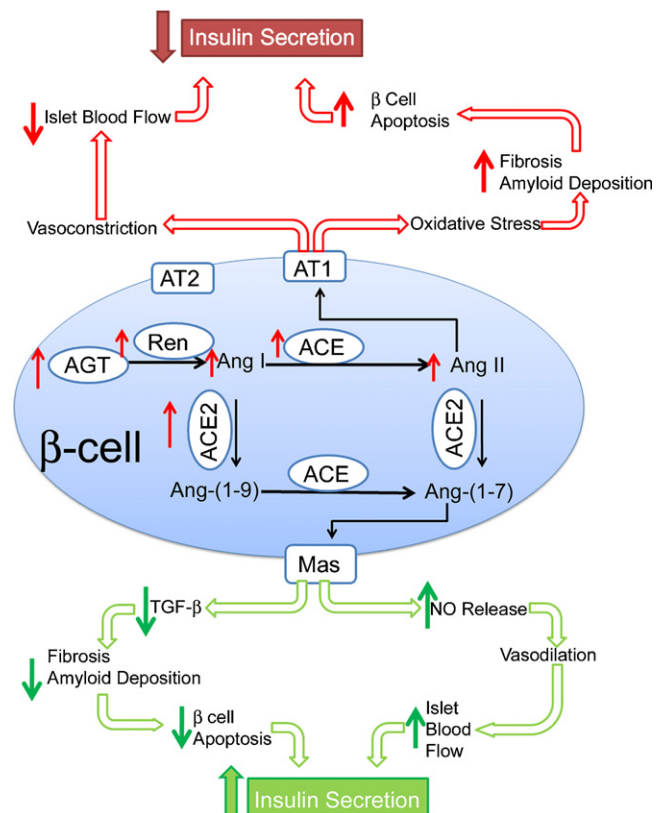


Fig. 3. Hypothetical model for the role of ACE2 in the pancreas. Ang-II is known to decrease islet blood flow and increase islet oxidative stress, which can cause β -cell apoptosis and decrease insulin secretion. The red arrows emphasize hyperglycemia-induced changes in the islet RAS and its consequences. ACE2 through TGF- β inhibition may reduce amyloid deposition, islet fibrosis and subsequent β -cell apoptosis. Ang-(1–7)-mediated vasodilation may lead to increased blood flow. The combined reduction in β -cell apoptosis and increase in islet blood flow could cause an increase in insulin secretion and preservation of islet function in diabetes. The green arrows highlight hypothetical pathways by which ACE2 may influence islet function.

Ang-II cleavage by ACE2, mediates vasodilation and therefore may enhance insulin sensitivity. In conclusion, recent data from the literature and our laboratory suggest that ACE2 expression is modulated during diabetes and that ACE2 deletion affects glucose homeostasis.

9. Perspectives

Several transgenic models are already in the pipeline to further address the role of ACE2 in diabetes and metabolic diseases and future studies are likely to confirm ACE2 as a new target for the treatment and prevention of diabetes and its complications. Animal models lacking ACE2 can be useful to demonstrate the role of ACE2 in the development of diabetes and diabetic complications. Accordingly, the breeding of ACE2^{-/-} with Akita mice has already revealed the importance of ACE2 in the prevention of diabetic kidney disease (Wong et al., 2007). Similar strategies involving ACE2 knockout mice and other diabetic models could further our understanding of the role of ACE2 in the development of diabetes and other diabetic complications. Additionally, DX600 (Huang et al., 2003) and MLN-4760 (Tikellis et al., 2003), two inhibitors of ACE2, have both been shown to be effective at reducing the effects of the enzyme. These compounds could be delivered to animals prior to or after the onset of diabetes to demonstrate the influence of ACE2 or Ang-(1–7) on the development of diabetes or diabetic complications.

Enhancing ACE2 through pharmacological or genetic intervention is also an attractive option for the prevention and treatment of diabetes and diabetic complications. Ferreira et al., recently demonstrated the efficacy of 2 pharmacological activators of ACE2, among which 1-[[2-(dimethylamino)ethyl]amino]-4-(hydroxymethyl)-7-[[[(4-methylphenyl)sulfonyl]oxy]-9H-xanthen-9-one (XNT) is capable of reducing cardiac fibrosis and high blood pressure in spontaneously hypertensive rats (Ferreira and Raizada, 2008). Finally, ACE2 viral delivery, and therefore over-expression, has been shown to reduce Ang-II-mediated responses (Feng et al., 2008; Huentelman et al., 2005).

In addition to the modulation of ACE2, direct targeting of Ang-(1–7) is also possible. To this end, the Ang-(1–7) receptor antagonist, D-Ala⁷-Ang-(1–7) (Santos et al., 1994), and the Ang-(1–7) analogue, AVE 0991 (Wiemer et al., 2002), are available. Recently, Shao et al. demonstrated that Ang-(1–7) infusion worsens diabetic kidney disease, which seems to oppose the idea that ACE2 is beneficial in diabetic kidney disease (Shao et al., 2008). These findings highlight the need for further study of the Ang-(1–7) signaling pathways and other potential effects of ACE2. The combination of the different strategies could help to discriminate between the effects of Ang-(1–7) generation and ACE2 activation in the development of diabetes and clarify the role of the ACE2/Ang-(1–7)/Mas receptor axis in this fast-progressing disease.

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