Angiotensin-converting enzyme 2 mediates hyperfiltration associated with diabetes

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Tikellis C, Brown R, Head GA, Cooper ME, Thomas MC. Angiotensin-converting enzyme 2 mediates hyperfiltration associated with diabetes. Am J Physiol Renal Physiol 306: F773–F780, 2014. First published January 29, 2014; doi:10.1152/ajprenal.00264.2013.—The degradation of ANG II by angiotensin-converting enzyme 2 (ACE2), leading to the formation of ANG(1–7), is an important step in the regulation of the renin-angiotensin-aldosterone system (RAAS), and one that is significantly altered in the diabetic kidney. This study examined the role of ACE2 in the hyperfiltration associated with diabetes. Streptozotocin diabetes was induced in male C57BL6 mice and ACE2 knockout (KO) mice. C57BL6 mice were further randomized to receive the selective ACE2 inhibitor MLN-4760. After 2 wk of study, animals were subjected to micropuncture experiments. The renal reserve was further assessed in C57BL6 mice and ACE2 KO mice after exposure to a high-protein diet. The induction of diabetes in wild-type mice was associated with increased renal ACE2 activity, hyperfiltration, and renal hypertrophy. On micropuncture, diabetes was associated with increased tubular free flow and stop-flow pressure, enhanced tubuloglomerular feedback reactivity, and an increased maximal response indicative of increased glomerular hydrostatic capillary pressure. Each of these increases were prevented in diabetic ACE2 KO mice and diabetic mice treated with a selective ACE2 inhibitor for 2 wk. However, unlike chronically treated animals, ACE2 inhibition with MLN-4760 had no acute effect on stop-flow pressure or tubuloglomerular feedback reactivity. ACE2 KO mice also failed to increase their creatinine clearance in response to a high-protein diet. The results of our study suggest that ACE2 plays a key role in the recruitment of the renal reserve and hyperfiltration associated with diabetes.

angiotensin; angiotensin-converting enzyme 2; hyperfiltration; diabetes; diabetic nephropathy; micropuncture

Hyperfiltration and the associated increases in glomerular capillary pressure are among the earliest renal changes linked to the initiation and progression of diabetic kidney disease (6). According to the “tubulocentric” hypothesis, glomerular hyperfiltration and hemodynamic dysfunction associated with diabetes are driven by tubular hypertrophy, which leads to increased proximal Na+ reabsorption and Na+-glucose cotransport, reduced distal Na+ delivery, and the subsequent activation of tubuloglomerular feedback (TGF) (42). Inhibition of tubular growth (36), reducing tubular glucose uptake (37), or increasing Na+ intake (41) are able to attenuate diabetes-associated hyperfiltration by increasing distal Na+ delivery. At the same time, the sensitivity of TGF may be substantially modified by diabetes (42). ANG II is a cofactor for the elicitation of TGF responses, in both the presence and absence of diabetes (18). Peritubular perfusion of ANG II enhances TGF responses (14), whereas TGF responses do not occur in ANG II type 1A (AT1A) receptor-deficient mice (7, 27) or in the absence of endothelial angiotensin-converting enzyme (ACE) (10).

In the diabetic kidney, the generation of ANG II from ANG I is determined by ACE as well as by ACE-independent pathways, including chymase. However, the degradation of ANG II is largely mediated by the zinc-dependent carboxypeptidase ACE2 (12). This places ACE2 as a potentially important regulator of ambient ANG II levels in the kidney. Moreover, the degradation of ANG II by ACE2 leads to the formation of ANG(1–7), which has a range of actions that oppose and counterbalance those of ANG II. ANG(1–7) is a vasodilator, with direct effects on Na+ balance, tubular growth, the production of nitric oxide, and prostaglandin synthesis (5, 13, 25). The renal vasculature is also known to be sensitive to the vasodilator effects of ANG(1–7), particularly at the afferent arteriole (22), and in states associated with activation of the intrarenal renin-angiotensin-aldosterone system (RAAS), such as diabetes (1).

The renal expression of ACE2 is significantly modified in the kidney after the induction of diabetes, especially in the proximal tubule, where the activity of ACE2 is increased (17, 31, 38, 44, 45), possibly as a compensatory (feedback) response to increased activation of the intrarenal RAAS in the diabetic kidney (45). In contrast, most studies (15, 21, 31, 46) have shown decreased intraglomerular expression of ACE2 associated with diabetes, whereas vascular ACE2 activity is also reduced (33), potentially contributing to the elevated circulating levels of ANG II and reduced levels of ANG(1–7) in experimental models of diabetes. In so far as hyperfiltration may also be considered a feedback compensation, and the RAAS clearly plays a role in this process, we hypothesized that changes in the expression of ACE2 may also play a role in the induction and maintenance of the hyperfiltration associated with diabetes.

MATERIALS AND METHODS

Induction of experimental type 1 diabetes. Wild-type male C57BL6 mice and ACE2 knockout (KO) mice [kindly donated by Dr. Penninger (4)] at 10 wk of age were randomly allocated to receive streptozotocin (55 mg/kg, Sigma Chemical, St. Louis, MO) or buffer (sodium citrate buffer, pH 4.5) delivered in five consecutive daily doses. This regimen induces a well-characterized insulinopenic form of diabetes associated with hyperglycemia (blood glucose ~30 mM) but with sufficient β-cell reserve to prevent ketosis or require insulin supplementation. Animals were then followed for 2 wk. Throughout the study, animals were given access to food and water ad libitum. Wild-type mice with and without diabetes were further randomized to receive the selective ACE2 inhibitor MLN-4760. For chronic dosing, we used 10 mg·kg−1·day−1 in drinking water, a dose we have...
previously shown to be effective at reducing intrarenal ANG(1–7) and preventing hyperfiltration (38). For acute dosing during micropuncture experiments, a dose of 100 μg/kg iv was used.

**High-protein diet.** Wild-type male C57BL6 mice and ACE2 KO mice at 10 wk of age were randomly allocated to receive normal chow or an isocaloric diet in which the protein concentration was increased to 52% (SF-0012, Speciality Feeds) for 2 wk.

**General assessment parameters.** Blood glucose levels were measured using a glucometer (Accutrend, Boehringer Mannheim, Mannheim, Germany), and glycated hemoglobin was measured by HPLC (CLC330 GHb Analyzer, Primus, Kansas City, MO) (3). Urine was collected from animals that had been placed in individual metabolic cages (Iffa Credo, L’Arbresle, France) for 24 h, allowing measurement of creatinine clearance, which was estimated from serum and urine creatinine concentrations determined by cation exchange HPLC (34). Blood pressure was measured by radiotelemetry (TA11PA-C20, Data Sciences) in a subset of animals as previously described (39).

Endogenous hippurate clearance, a marker of renal plasma flow, was estimated using HPLC, as previously described (34). All experiments were approved by the Animal Ethics Committee of the Alfred medical research precinct and were conducted in an Institutional Animal Care and Use Committee-approved facility in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

**Disposition of mice.** After 2 wk of study, mice were culled by a fatal overdose of anesthetic followed by cardiac exanguination. The kidneys were rapidly dissected out. Each kidney was cleaned of fat and weighed, with the result expressed as the combined weight adjusted for body surface area (in g/m²). Kidneys were then bisected, with one half snap frozen in liquid nitrogen and stored at −80°C before use. To estimate the degree of tubular hypertrophy in our model systems, the mean value of the shortest diameter of 100 proximal tubules was measured in periocid acid Schiff-stained kidneys using the Optimas 6.2 image-analysis system, as previously described (35).

**RAAS and ACE2 activity.** Gene expression of various components of the renin-angiotensin-aldosterone system, including ACE, ACE2, and prorenin mRNA in cortical homogenates, were assessed by real-time quantitative RT-PCR, as previously described (38). The intrarenal concentration of ANGII and ANG(1–7) was measured in unpaired frozen renal cortical samples by a commercial radioimmunoassay (ProSearch, Malvern, Victoria, Australia) using an ANG(1–7)-selective polyclonal antibody and 125I-labeled ANG(1–7). Before analysis, samples were homogenized in ice-cold methanol in the presence of protease inhibitors (50 mM EDTA, 0.5 mM iodoacetamide, 1 mM phenylmethyl sulfonyl fluoride, and 0.1 mM pepstatin A). Samples were then centrifuged to remove protein, and the upper (aqueous) phase was removed and lyophilized in glass tubes at −80°C. Results are expressed adjusted for the wet weight of the sample. ACE2 activity was determined after incubation with the intramolecularly quenched synthetic ACE2-specific substrate α-aminoacetic acid-Ser-Pro-Tyr(NO2)OH (Peptides), as previously described (38).

**Micropuncture experiments.** A previous study (42) has suggested that hyperfiltration in diabetes may be initiated by increased proximal tubular fluid reabsorption combined with decreased sensitivity of TGF. To test the role of ACE2 in these phenomena, TGF characteristics were determined as detailed below. Mice were anesthetized by spontaneous inhalation of 2.2% isoflurane and placed on a servo-regulated heating pad to maintain body temperature at 37.5°C. Catheters were inserted into the carotid artery and jugular vein for blood pressure measurements and the infusion of maintenance fluid (0.9% NaCl, 0.35 ml/h), respectively. The left kidney was exposed through a subcostal flank incision, dissected free from surrounding tissue, placed in a Lucite cup, and fixed in a 3% agar solution. TGF characteristics were determined by the stop-flow technique as previously described (2). Proximal tubular free-flow pressure was measured in randomly chosen superficial segments with a pipette filled with a 1 M NaCl solution and connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT). In nephrons in which three or more tubular segments were identified, tubular flow was interrupted with a wax block. A pipette containing artificial tubular ultrafiltrate and connected to a microperfusion pump (Hampel, Frankfurt, Germany) was inserted into the last accessible tubular segment. Stop-flow pressure (PSFw) was determined upstream to the block at various perfusion rates between 0 and 35 nl/min by changing the perfusion rate by 2.5- to 5-nl increments. The maximal change in PSF (ΔPSFmax) was determined as the difference in PSFw when the perfusion rate was increased from 0 to 35 nl/min. This parameter was used to estimate TGF reactivity. The tubular perfusion rate eliciting half-maximal ΔPSFw, the turning point (TP), was determined and served to indicate TGF sensitivity. The response curves shown in Fig. 4 were plotted using a previously described normalization method (28). Normalized data were fitted to the following equation by means of a nonlinear least-squares curve-fitting program (Minuit): PSFw = PSFmin + ΔPSF/1 + exp^{-PR - TP}, where ΔPSFw is the average maximal PSFw response, PSFmin is the average minimum PSFw when the distal perfusion is increased, w is the factor determining the width of the perfusion interval during which the PSFw responded, and PR is the end-proximal perfusion rate.

**Statistical analysis.** Continuous data are expressed as means ± SE. Differences in the mean among groups were compared using two-way ANOVA with diabetic status and ACE2 status as the two variables. Pairwise multiple comparisons were made with Student-Newman-Keuls post hoc analysis to detect significant differences between groups. P values of <0.05 were considered statistically significant.

**RESULTS**

**General parameters.** The induction of streptozotocin diabetes was associated with a significant increase in plasma glucose concentration (~25–30 mM) and glycated hemoglobin compared with respective nondiabetic controls (Table 1). This increase associated with diabetes was similar in wild-type and ACE2 KO mice and in mice treated with the selective ACE2 inhibitor MLN-4760 (10 mg·kg⁻¹·day⁻¹). Genetic ACE2 deficiency was associated with a modest increase in mean arterial blood pressure (MABP) compared with wild-type mice, as measured by radiotelemetry (Fig. 1) and plethysmography using a tail cuff (Table 1). The induction of diabetes was associated with a small but significant fall in MABP that was proportionally greater in ACE2 KO mice than in wild-type mice, so that the difference in MABP between strains was eliminated in diabetic mice (Fig. 1). The ACE2 inhibitor had no significant effect on blood pressure levels in the presence or absence of diabetes (Table 1).

**Changes in the RAAS.** Diabetes was also associated with an increase in the renal cortical activity of ACE2 in diabetic mice (Fig. 2), as previously described (17, 31, 38, 44, 45). Plasma ACE2 activity was also increased in diabetic mice (24 ± 2 ngeq/ml) compared with nondiabetic mice (15 ± 3 ngeq/ml). However, despite this increase in angiotensinase activity, the induction of diabetes in C57BL6 mice was also associated a fall in renal cortical levels of ANG(1–7), whereas renal cortical and circulating levels of ANG II were increased in diabetic mice (Fig. 2). One week of diabetes was also associated with changes in the renal cortical expression of ACE, (pro)renin, AT1 receptor, and ANG II type 2 (AT2) receptor mRNA (Table 2). ACE2 KO mice also had reduced ANG(1–7) levels and increased tissue and circulating levels of ANG II, but this did not change after the induction of diabetes, as previously described (38, 39). No gene or protein expression of ACE2 or
ACE2 activity was observed in ACE2 KO mice. The induction of renal cortical expression of (pro)renin and the AT1 receptor associated with diabetes were attenuated in ACE2 KO mice (Table 2), whereas changes in the AT2 receptor were unassociated with diabetes were attenuated in ACE2 KO mice (Fig. 1). Circulating ACE2 activity was also reduced by chronic ACE2 inhibition to 23% of that observed in wild-type mice and 25% of that observed in diabetic mice. However, we were not able to detect any significant effect of chronic ACE2 inhibition on ACE2 activity in the cortex (Fig. 2).

ACE2 deficiency and hyperfiltration in diabetic mice. The induction of diabetes in C57BL6 mice was associated with a significant increase in creatinine clearance compared with nondiabetic mice (Table 1). These increases were attenuated in diabetic ACE2 KO mice and in diabetic mice receiving the selective ACE2 inhibitor MLN-4760. The induction of diabetes was also associated with an increase in renal plasma flow that was also attenuated in diabetic ACE2 KO mice and in mice that received the selective ACE2 inhibitor MLN-4760 (Table 1). Renal function was not modified in nondiabetic ACE2 KO mice or after treatment of wild-type mice with a selective ACE2 inhibitor (Table 1).

One of the earliest structural changes in the diabetic kidney is hypertrophy and hyperplasia of the cortical tubuli leading to renal enlargement (32). In our study, the induction of diabetes in C57BL6 mice was associated with a rapid increase in tubular diameter (as a marker of proximal tubular hypertrophy) and surface area-adjusted renal mass (Table 1). The increase in renal mass after the induction of diabetes was attenuated (but not prevented) in diabetic ACE2 KO mice and in diabetic mice that chronically received the selective ACE2 inhibitor MLN-4760. However, no effect on tubular hypertrophy associated with diabetes was observed.

ACE2 and TGF characteristics. The induction of diabetes in wild-type mice was associated with increased tubular free-flow and P_{SF} and increased maximal response indicative of increased glomerular hydrostatic capillary pressure. Each of these increases were prevented in diabetic ACE2 KO mice and diabetic mice treated with a selective ACE2 inhibitor for 7 days (Table 3 and Fig. 3). The increase in intraglomerular pressure associated with diabetes is thought to have tubular and vascular components. The vascular response in diabetes is known to be enhanced. Consistent with this observation, we also found that TGF reactivity was enhanced in diabetic mice (i.e., the cumulative and maximal response was increased; Fig. 3). However, this increase was also prevented in diabetic ACE2 KO mice and in diabetic mice treated with a selective ACE2 inhibitor for 7 days (Fig. 3).

To explore whether the effects of ACE2 deficiency represent the acute dysregulation of renal hemodynamics or chronic changes associated with changes in the intrarenal RAAS, diabetic mice were treated acutely with an intravenous ACE2 inhibitor. However, unlike chronically treated animals, ACE2 inhibition with intravenous MLN-4760 had no acute effect on P_{SF} (Table 3) or TGF reactivity (Fig. 3).

**Table 1. General and renal parameters**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Glucose, mmol/l</th>
<th>Glycated Hemoglobin, %</th>
<th>Blood Pressure, mmHg</th>
<th>Renal Mass, g/m²</th>
<th>Proximal Tubular Diameter, μm</th>
<th>Creatinine Clearance, ml·min⁻¹·m⁻²</th>
<th>Effective Renal Plasma Flow, ml·min⁻¹·m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6 (wild-type) mice</td>
<td>33 ± 1</td>
<td>10.1 ± 1.2</td>
<td>4.1 ± 0.1</td>
<td>103/85</td>
<td>37 ± 1</td>
<td>36 ± 1</td>
<td>26 ± 3</td>
<td>70 ± 20</td>
</tr>
<tr>
<td>ACE2 KO mice</td>
<td>31 ± 1</td>
<td>9.1 ± 1.1</td>
<td>3.5 ± 0.2</td>
<td>113*85</td>
<td>36 ± 1</td>
<td>35 ± 1</td>
<td>33 ± 2</td>
<td>130 ± 12*</td>
</tr>
<tr>
<td>C57BL6 mice + diabetes</td>
<td>26 ± 1*</td>
<td>30.1 ± 1.3*</td>
<td>12.0 ± 0.6*</td>
<td>96<em>81</em></td>
<td>45 ± 1*</td>
<td>42 ± 1*</td>
<td>66 ± 5*</td>
<td>210 ± 21*</td>
</tr>
<tr>
<td>ACE2 KO mice + diabetes</td>
<td>24 ± 1*</td>
<td>29.1 ± 1*</td>
<td>11.9 ± 0.6*</td>
<td>102/76*</td>
<td>41 ± 1†</td>
<td>41 ± 1*</td>
<td>45 ± 3*</td>
<td>120 ± 13†</td>
</tr>
<tr>
<td>Diabetic mice + chronic ACE2 inhibitor</td>
<td>28 ± 2*</td>
<td>29.2 ± 1.2*</td>
<td>13.7 ± 0.8*</td>
<td>98<em>80</em></td>
<td>42 ± 1†</td>
<td>42 ± 1*</td>
<td>37 ± 2†</td>
<td>110 ± 12††</td>
</tr>
<tr>
<td>Diabetic mice + acute ACE2 inhibitor</td>
<td>28 ± 2*</td>
<td>30.1 ± 1.3*</td>
<td>13.2 ± 0.8*</td>
<td>100<em>78</em></td>
<td>45 ± 1†</td>
<td>43 ± 1*</td>
<td>67 ± 6†‡</td>
<td>213 ± 17††</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 mice/group. ACE2, angiotensin-converting enzyme 2; KO, knockout. *P < 0.05 vs. control (wild-type) mice; †P < 0.05 vs. diabetes alone. §Before exposure to the ACE2 inhibitor.
Na\(^+\) and activation of TGF. Elevated levels of ANG II are also thought to play an important role in protein-mediated hyperfiltration (9). However, unlike diabetes, the sensing mechanism in the TGF system is not altered by manipulation of dietary protein (29). In wild-type C57BL6 mice, exposure to a high-protein diet (52% protein) was associated with an increase in renal mass and an increase in creatinine clearance (Fig. 4). The renal expression of (pro)renin and the producer of vasodilatory prostaglandins cyclooxygenase 2 were also greater in mice that received a high-protein diet compared with normal chow (20% protein; Fig. 4). In addition, intrarenal ACE2 activity was increased in wild-type mice exposed to a high-protein diet compared with standard chow (18 ± 3 ngeq/mg, \(P < 0.05\)). There was no change in plasma ACE2 activity. Exposure of ACE2 KO mice to a high-protein diet was also associated with renal hypertrophy and the induction of (pro)renin and cyclooxygenase-2. However, hyperfiltration was attenuated in ACE2 KO mice, implying a functional loss of the renal reserve.

**DISCUSSION**

Hemodynamic changes in the kidney associated with uncontrolled hyperglycemia are well known and include an exaggerated response in P\(_{SF}\), hyperfiltration, and increased intraglomerular pressure (6). However, the precise mediators of these processes continue to be a matter of ongoing research (42). In this study, we demonstrate that ACE2 KO mice and mice in which ACE2 activity has been chronically inhibited lack the ability to develop hyperfiltration or increase glomerular hydrostatic capillary pressure in response to diabetes. ACE2 KO mice also do not hyperfilter in response to a high-protein diet, suggesting that changes in the expression or activity of ACE2 may be important for recruitment of the renal reserve in diabetic mice.

Degradation of ANG II in the kidney is largely mediated by the zinc-dependent carboxypeptidase ACE2 (12). Although the diabetic kidney is generally regarded to suffer functional RAAS overactivity, the intrarenal activity of ACE2 protein is

![Fig. 2. Changes in the renin-angiotensin-aldosterone system induced in ACE2 KO mice and mice treated chronically with an ACE2 inhibitor in the presence and absence of diabetes, including cortical ANG II (A), cortical ANG(1–7) (B), plasma ANG II (C), and cortical ACE2 activity (D). *\(P < 0.05\) vs control.](http://ajprenal.physiology.org/)

**Table 2. Renal cortical gene expression of components of the renin-angiotensin system relative to control**

<table>
<thead>
<tr>
<th></th>
<th>ACE2</th>
<th>ACE</th>
<th>Prorenin</th>
<th>ANG II Type 1 Receptor</th>
<th>ANG II Type 2 Receptor</th>
<th>Mas Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6 (wild-type) mice</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>ACE2 KO mice</td>
<td>N/A</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>0.6 ± 0.2*</td>
</tr>
<tr>
<td>C57BL6 mice + diabetes</td>
<td>1.1 ± 0.1</td>
<td>0.3 ± 0.1*</td>
<td>2.6 ± 0.7*</td>
<td>2.1 ± 0.3*</td>
<td>2.0 ± 0.3*</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>ACE2 KO mice + diabetes</td>
<td>N/A</td>
<td>0.2 ± 0.1*</td>
<td>0.9 ± 0.1*†</td>
<td>0.9 ± 0.1*†</td>
<td>1.7 ± 0.5*</td>
<td>0.8 ± 0.1†</td>
</tr>
<tr>
<td>Diabetic mice + chronic ACE2 inhibitor</td>
<td>0.6 ± 0.1*†</td>
<td>0.3 ± 0.1*</td>
<td>0.7 ± 0.1*†</td>
<td>0.7 ± 0.1*†</td>
<td>1.9 ± 0.3*</td>
<td>0.6 ± 0.1*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 8\) mice/group. N/A, not applicable. *\(P < 0.05\) vs. control (wild-type) mice; †\(P < 0.05\) vs. C57BL6 mice + diabetes.
increased in experimental diabetes (Fig. 2) (17, 31, 38, 44, 45) and in renal biopsy samples of patients with diabetic kidney disease (11). This increase in renal ACE2 may represent a futile attempt to normalize ANG II and ANG(1–7) levels against a diabetes-associated increase in ANG II reduction and fall in ANG(1–7). Thus, ACE2 could exert a protective effect against the development of early nephropathy (45) and may be one reason why ACE2 deficiency or inhibition results in accelerated renal damage associated with diabetes (17, 31, 38, 44, 45). However, the same compensatory changes in the intrarenal RAAS determined by ACE2 also appear to contribute to hyperfiltration, which is generally thought to be causally linked to glomerular injury in diabetes (6). Taken together, these data suggest that the role of ACE2 is more complicated than simply “a good or bad ACE.”

In response to hyperglycemia or a high-protein diet, renal blood flow is enhanced with dilation of resistance arterioles. A number of factors contribute to this change, including the RAAS. It has been suggested that diabetes is associated with an impaired reactivity to exogenous ANG II (8). As the carboxypeptidase ACE2 is able to break down ANG II, the increased angiotensinase activity described in the blood and kidneys of our diabetic mice may provide one explanation for this phenomenon. In addition, the renal vasculature is also highly sensitive to the vasodilator effects of ANG(1–7) generated by ACE2, particularly at the afferent arteriole (22), and in states associated with activation of the local RAAS. For example, glomerular filtration is substantially enhanced after an infusion of ANG(I-7) in anesthetised rats where RAAS activity is enhanced by placing them on a low-salt diet (16). Similarly, ANG(1–7) appears to have more potent vasodilator effects in the diabetic vasculature (1). Given that ACE2 locally generates ANG(1–7), it is plausible that any intrarenal increase in ACE2 activity may also contribute to hyperfiltration.

Table 3. Renal micropuncture parameters

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP, mmHg</th>
<th>P_{Ty}, mmHg</th>
<th>P_{SF}, mmHg</th>
<th>ΔP_{SF}, mmHg</th>
<th>TP, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL6 (wild-type) mice</td>
<td>93 ± 2</td>
<td>11.8 ± 0.8</td>
<td>37.5 ± 0.4</td>
<td>6.9 ± 0.4</td>
<td>193 ± 0.6</td>
</tr>
<tr>
<td>ACE2 KO mice</td>
<td>91 ± 1</td>
<td>12.0 ± 0.3</td>
<td>38.1 ± 0.4</td>
<td>7.3 ± 0.6</td>
<td>204 ± 1.0</td>
</tr>
<tr>
<td>C57BL6 mice + diabetes</td>
<td>91 ± 2</td>
<td>13.2 ± 0.5*</td>
<td>38.7 ± 0.6*</td>
<td>11.7 ± 1.4*</td>
<td>185 ± 0.9</td>
</tr>
<tr>
<td>ACE2 KO mice + diabetes</td>
<td>93 ± 1</td>
<td>12.9 ± 0.6</td>
<td>38.4 ± 0.9*</td>
<td>7.6 ± 0.7†</td>
<td>193 ± 0.8</td>
</tr>
<tr>
<td>Diabetic mice + chronic ACE2 inhibitor</td>
<td>92 ± 3</td>
<td>11.8 ± 0.3†</td>
<td>38.8 ± 0.4*</td>
<td>7.8 ± 1.4†</td>
<td>196 ± 1.0</td>
</tr>
<tr>
<td>Diabetic mice + acute ACE2 inhibitor</td>
<td>90 ± 2</td>
<td>13.5 ± 0.5*</td>
<td>38.4 ± 0.7*</td>
<td>11.3 ± 0.8*</td>
<td>186 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 mice/group. MAP, mean arterial pressure during the procedure; P_{Ty}, tubular free-flow pressure; P_{SF}, stop-flow pressure; ΔP_{SF}, change in P_{SF}; TP, turning point. *P < 0.05 vs. control (wild-type) mice; †P < 0.05 vs. diabetes alone.
It has been argued that hyperfiltration in diabetes may be driven by tubular hypertrophy and result in increased proximal tubular Na\(^+\) uptake. Certainly, inhibition of tubular growth in diabetes may attenuate hyperfiltration (36). However, blockade of the RAAS does not prevent renal hypertrophy in diabetes or after exposure to a high-protein diet (30). Similarly, in our model, deletion of ACE2 failed to prevent tubular hypertrophy in diabetes (Table 1) and the increase in renal mass after exposure to a high-protein diet (Fig. 4), although total renal mass was modestly reduced after the induction of diabetes in ACE2 KO mice (Table 1). Taken together, these data suggest that the failure to recruit renal function in ACE2 KO mice is not related to reduced tubular hypertrophy.

Some previous studies (14, 20) have suggested that the TGF response is augmented by intravenous or peritubular infusions of ANG II. Despite altered intrarenal concentrations of ANG II and ANG(1–7), ACE2 KO mice have normal renal function, normal TGF, and normal renal pressures. At this age, their kidneys also appear healthy, with no evidence of glomerulosclerosis or nephron drop out. These findings suggest that ACE2 is not an important regulator of normal renal function or nephrogenesis. However, on the basis of our findings, it is possible to suggest that ACE2 plays a role in augmenting renal blood flow and recruiting the renal reserve. It is possible that ANG II modulates the renovascular actions of adenosine, meaning that some other stimulus (like diabetes or a high-protein diet) is required to unmask the effects of elevated ANG II (26). Notably, ACE2 inhibition did not acutely reverse hyperfiltration or modify glomerular pressure changes associated with diabetes, although 2 wk of treatment was able to prevent it. This further suggests that the actions of ACE2 in mediating hyperfiltration are more chronic, possibly involving nitric oxide synthesis or vascular sensitivity.

It has recently been argued that both diabetes and high protein-induced hyperfiltration are independent of the TGF mechanism, as adenosine A\(_1\) receptor KO mice, which lack a functional (acute) TGF mechanism, still display pronounced hyperfiltration when diabetes is induced (23) or with a high-protein diet (24). However, another study (40) has observed that hyperfiltration is blunted in adenosine A\(_1\) receptor KO diabetic mice, potentially validating the role of TGF in diabetes-associated hyperfiltration. Moreover, acute hyperglycemia increases the glomerular filtration rate in dogs, but only if TGF is intact (43). TGF is a homeostatic control mechanism designed to stabilize NaCl delivery into the late part of the nephron and thereby stabilize NaCl excretion. In our study, we observed that the induction of diabetes in wild-type mice was associated with increased TGF reactivity, increased tubular free-flow pressure and \(P_{SF}\), and an increased maximal \(P_{SF}\) response indicative of increased glomerular hydrostatic capillary pressure. However, the intrinsic sensitivity of TGF was not different between control and diabetic mice, as reflected by the lack of horizontal displacement (left-right shift) in the TGF pressure-flow curves. This is consistent with findings in other rodent models of experimental diabetes. Notably, in our mouse model, insulin is not required to prevent ketosis or maintain animal health. This may be important as streptozotocin-induced diabetes in rats produces an insulinopenic diabetes in which...
renal hemodynamic changes may be substantially altered by the presence of even small amounts of insulin (19).

While a number of studies have promoted the potential renoprotective utility of recombinant ACE2 or ANG(1–7) agonists in diabetic nephropathy, these findings suggest that the actions of the RAAS in the diabetic kidney are more complicated than too much ANG II. This may be one reason why conventional RAAS blockade has not provided the optimal renoprotection desired for our patients with diabetes. Indeed, by reducing ANG II synthesis and reducing ANG(1–7) degradation, ACE inhibition achieves much the same actions as ACE2 excess and, consequently, does not attenuate hyperfiltration associated with diabetes.

In summary, the results of our study suggest that ACE2 plays a key role in the recruitment of the renal reserve and hyperfiltration associated with diabetes or a high-protein diet. These findings provide further evidence for the pivotal actions of the RAAS in renal function and add to the growing complexity of our knowledge of the intrarenal RAAS.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.T., M.E.C., and M.C.T. conception and design of research; C.T., R.B., G.A.H., and M.C.T. performed experiments; C.T. and M.C.T. interpreted results of experiments; C.T., R.B., G.A.H., and M.C.T. approved final version of manuscript; R.B. and M.C.T. analyzed data; M.C.T. prepared manuscript; R.B. and M.C.T. revised manuscript; C.T., R.B., G.A.H., and M.C.T. performed experiments; C.T. and M.C.T. analyzed data; M.C.T. contributed unpublished data; M.C.T. approved final version of manuscript;

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