Renal prostaglandin E2 receptor (EP) expression profile is altered in streptozotocin and B6-Ins2Akita type I diabetic mice

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Nasrallah R, Xiong H, Hébert RL. Renal prostaglandin E2 receptor (EP) expression profile is altered in streptozotocin and B6-Ins2Akita type I diabetic mice. Am J Physiol Renal Physiol 292: F278–F284, 2007. First published September 5, 2006; doi:10.1152/ajprenal.00089.2006.—The homeostatic function of prostaglandin E2 (PGE2) is dependent on a balance of EP receptor-mediated events. A disruption in this balance may contribute to the progression of renal injury. Although PGE2 excretion is elevated in diabetes, the expression of specific EP receptor subtypes has not been studied in the diabetic kidney. Therefore, the purpose of this study was to characterize the expression profile of four EP receptor subtypes (EP1–4) in 16-wk streptozotocin (STZ) and B6-Ins2Akita type I diabetic mice. In diabetic mice, the ratio of kidney weight to body weight was increased twofold compared with controls, blood glucose was elevated, but urine albumin was only increased in B6-Ins2Akita mice. The excretion of PGE2 and its metabolite was augmented two- to fourfold as determined by enzyme immunoassay. Accordingly, renal cyclooxygenases were also increased in diabetic mice, with isoform-specific and regional differences in each model. Finally, there was altered EP1,4 receptor expression in diabetic kidneys, with significant differences between STZ and B6-Ins2Akita mice (fold-control). In STZ mice, cortical EP1 increased by 1.6, EP2 increased by 2.3, and EP4 decreased by 0.63; yet in B6-Ins2Akita mice, cortical EP1 increased by 2.4, but there was a general decrease in the remaining subtypes. Similarly, in the STZ medulla EP3 increased by 3.6, but both EP1 and EP3 increased by 5.5 and 1.95, respectively, in B6-Ins2Akita mice. Therefore, knowing the pattern of change in relative EP receptor expression in the kidney could be useful in identifying specific EP targets for the prevention of various components of diabetic kidney disease.

cyclooxygenase; real-time reverse transcriptase polymerase-chain reaction; renal EP receptors; streptozotocin-diabetic mice

DIABETIC NEPHROPATHY (DN) is a leading cause of chronic kidney disease resulting in end-stage renal disease (ESRD). The renal changes associated with DN consist of glomerular, vascular, and tubular events that result in altered renal hemodynamics, growth responses, matrix accumulation, and tubular transport processes. To date, the most common therapy for management of the disease, aimed at slowing the development of diabetic complications and ESRD, consists of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists, which reduce blood pressure and delay the progressive loss of renal function (4). Despite this intervention, diabetes is threatening to reach epidemic proportions, and current modes of therapy are ineffective in preventing this course. While glucose is the main determinant of the changes associated with the pathogenesis of DN, the induction of numerous downstream effectors results in a vicious cycle of events, all uniting to perpetuate the extent of renal injury. It is therefore imperative to clarify the specific disturbances underlying the injury to the kidney, to find alternative targets for more specific therapeutic intervention.

Prostaglandin E2 (PGE2) is by far the most predominant arachidonic acid metabolite produced in the kidney (6), particularly in the glomerular regions and the inner medulla. Its synthesis is dependent on the activity of two cyclooxygenase isoforms (COX-1 and COX-2) that are inhibited by nonsteroidal anti-inflammatory drugs (NSAIDS). PGE2 is important in various aspects of renal physiology, hemodynamics, renin release, and tubular transport processes. Its biological effects are mediated by four distinct G protein-coupled receptors, EP1–4 (32): the EP1 receptor stimulates intracellular calcium and activates protein kinase C via the Gq protein family; the EP2 receptor stimulates adenylate cyclase via Gs protein, and to date it remains unclear whether this receptor is expressed in renal tubules and what role it plays in renal physiology; the EP3 receptor inhibits adenylate cyclase via pertussis toxin-sensitive Gi protein, and by this mechanism PGE2 antagonizes arginine vasopressin (AVP) responses; and the EP4 receptor stimulates adenylate cyclase via Gi protein. The existence of various PGE2/EP receptor pathways in renal cells provides an interesting example of homeostatic processes, with redundant functions as well as opposing actions. This is exemplified by the fact that no obvious renal pathology or disturbance in function has been observed in any of the EP knockout mice (14, 23, 36, 41).

The past decade has led to many advances in the study of the effects of PGE2 through EP receptors in the kidney, but little is known about the pathophysiological role of each EP subtype in renal disorders and what effect a change in the relative responses in different EP pathways would have in a disease setting. While alterations in PG levels have been implicated in the pathogenesis of diabetic nephropathy (20, 27), resulting in hemodynamic changes and structural variations (13), the individual contribution of COX-1 and COX-2, as well as specific PG receptor pathways, to distinct aspects (altered H2O and Na+ balance, growth responses, matrix expansion, etc.) of diabetic kidney disease is lacking.

Therefore, we hypothesize that 1) an imbalance in the PGE2/EP system may be evident in diabetic kidneys and 2) this may contribute to renal injury in diabetes. Thus the purpose of this work addresses the first part of the hypothesis and examines the expression of four EP receptor subtypes in the different
regions of the kidney in 16-wk streptozotocin (STZ)-diabetic and B6-Ins2\(^{Akita}\) mice, two mouse models of type 1 diabetes. Identifying the relative expression of EP receptors should shed light on the usefulness of specifically targeting EP receptors to rectify an imbalance that may influence the evolution of diabetic nephropathy.

**MATERIALS AND METHODS**

Diabetic mouse models. Two separate studies were carried out using chemically induced-diabetic as well as genetically diabetic mice.

STZ-induced type 1 diabetes is the most widespread method of inducing type I diabetes in rodents by destroying the pancreatic \(\beta\)-cells (40). We administered 65 mg/kg of STZ/Na-citrate buffer (Sigma) three times daily intramuscularly, which induces diabetes in C57BL/6 mice within 1 wk after the infusion. Vehicle-treated controls were used for comparison. The major drawback with this model is the lack of similarity to human diabetes (15), with very mild changes reported in the kidney but also the known cellular toxicity of STZ (5). For this reason, a spontaneous type I diabetes model, the B6-Ins2\(^{Akita}\) mouse, was also studied.

The B6-Ins2\(^{Akita}\) model of spontaneous type I diabetes is a relatively new model of nonobese insulin-dependent diabetes, characterized by early-age onset and autosomal dominant inheritance. B6-Ins2\(^{Akita}\) mice were purchased from Jackson Laboratories and were derived from C57BL/6 mice, allowing for better comparisons in our studies, given the recognized strain differences in development of diabetic kidney changes (15). A mutation in the insulin 2-gene (Cys96Tyr) is responsible for their phenotype, showing progressive diabetes characterized by hyperglycemia (3) and notable pancreatic \(\beta\)-cell dysfunction. In a recent review by Breyer et al. (7) for the Animal Models of Diabetes Complications Consortium (AMDCC), the B6-Ins2\(^{Akita}\) model is reported as the optimal substitute for the well-established STZ diabetes model, to avoid issues of nonspecific cell toxicity and because it is commercially available through Jackson Laboratories. The homozygous mice die within 2 mo of age, but the male heterozygotes display diabetic symptoms including hyperglycemia (3) and notable pancreatic \(\beta\)-cell dysfunction. In a recent review by Breyer et al. (7) for the Animal Models of Diabetes Complications Consortium (AMDCC), the B6-Ins2\(^{Akita}\) model is reported as the optimal substitute for the well-established STZ diabetes model, to avoid issues of nonspecific cell toxicity and because it is commercially available through Jackson Laboratories.

Standard protocols were utilized for comparative studies to characterize the diabetic state of the mice, including kidney and body weight measurements, weekly determination of blood glucose levels using a blood glucose meter (Ascensia Elite, Bayer), systolic blood pressure measured by tail-cuff plethysmography (BP-2000, Visitech Systems), as well as urine albumin levels determined by enzyme-linked immunosorbent assay (Albuwell M competitive ELISA, Cedarlane Labs) and normalized by urine creatinine determination (colorimetric assay, Oxford Biomedical Research).

**Western blotting.** Protein lysates from cortex and medullary regions were prepared by homogenizing the tissue in RIPA buffer containing 1% NP-40, 1% sodium deoxycholate, 0.1% SDS (wt/vol), 4.5 mM NaCl, 2.5 mM Tris (pH 7.4), 8 μM EDTA, 0.2 mM sodium phosphate (pH 7.2), and freshly added 0.5 mM PMSF, 1:100 protease inhibitor cocktail (Sigma), 1 mM sodium pyrophosphate, 10 mM sodium fluoride, and 100 μM sodium orthovanadate. Twenty-five micrograms of each sample were resolved by SDS-PAGE on a polyacrylamide gel and transferred to a nitrocellulose membrane. After blocking for 2 h in 10% milk/TBS-T, the membranes were incubated overnight with either anti-COX-I (Santa Cruz) or anti-COX-2 (Cayman) polyclonal antibodies. Following incubation with a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody, enhanced chemiluminescence was used to visualize the signals. A single band of 65 or 72 kDa was obtained for COX-1 and COX-2, respectively. The samples were then normalized with detection of β-actin, and a densitometric analysis was performed using Kodak Digital Science 1D Image Analysis software (Eastman Kodak).

**Enzyme immunoassays.** Urine was collected from wild-type and diabetic mice at 16 wk of diabetes. The amount of PGE\(_2\) and its metabolite 11-deoxy-13,14-dihydro-15-keto-11\(\beta\), 16-\(\epsilon\)-cyclo-PGE\(_2\) (PGEM) was determined by competitive enzyme immunoassays (Cayman Chemical) following the manufacturer’s instructions. Briefly, the assay is based on a competitive binding of PGE\(_2\) or PGEM and their respective acetylcholinesterase conjugate (tracer) for a limited amount of monoclonal antibody. Since the tracer concentration is held constant, the amount of tracer bound to the antibody will be inversely proportional to the amount of PG in the sample. Detection is based on a colorimetric reaction using Ellman’s Reagent, which contains the substrate to acetylcholinesterase. The intensity is then determined by spectrophotometry. A colorimetric assay of urinary creatinine was performed for each sample to normalize the amount of PGE\(_2\) or PGEM.

**Real-time RT-PCR.** Total RNA was isolated using TRIzol (GIBCO) from different preparations of cortex and medulla from wild-type and diabetic mice at 16 wk of diabetes. The relative quantity of each target nucleic acid in different samples was determined by analyzing the cycle-to-cycle change in fluorescence signal as a result of amplification during a PCR. To quantify the amount of RNA in each sample, a relative standard curve was prepared by diluting a stock of control RNA. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA is detected as an internal control to standardize the amount of sample RNA added to a reaction. RT-PCR was performed using a 

**RESULTS**

Comparison of diabetic characteristics in 16 wk STZ and B6-Ins2\(^{Akita}\) mice. At 16 wk of age, STZ and B6-Ins2\(^{Akita}\) mice are hyperglycemic (see Tables 2 and 3), with significantly elevated blood glucose levels in the range of 25–30 mM. It has previously been reported that hyperglycemia is evident in both models at 4 wk of age (3, 28). Since renal hypertrophy is an important feature of diabetic kidneys, we observed an increase in kidney weight-to-body weight ratios in both groups of diabetic mice as expected. Urine albumin levels were elevated 3.8-fold in 16-wk B6-Ins2\(^{Akita}\) mice (Table 3). However, our work shows no differences in urine albumin in STZ mice (see Table 2), similar to recent reports by Gürley et al. (15). To the best of our knowledge, there have been no reports of changes in blood pressure in the STZ-diabetic mice, although we did not expect hypertension in these mice as early as 16 wk of age (Table 2); as shown in Table 3, we also did not detect any differences in blood pressures in B6-Ins2\(^{Akita}\) mice.
Renal COX-1 and COX-2 and urinary PGE2 are increased in diabetic mice. Our group and others have previously demonstrated that COX isoforms are elevated in diabetic kidneys (27, 33–35). As shown in Figs. 1 and 2, Western blot analysis confirms that COX isoforms are altered in both mouse models of type I diabetes. However, the following notable differences were observed. 1) In STZ mouse cortex, both COX-1 and COX-2 are increased 3.6- and 3-fold, respectively, but in B6-Ins2Akita mouse, a 2-fold increase is only seen for COX-1. 2) In the medulla, COX-1 is unchanged in the STZ mice and decreases to 0.14-fold of control in B6-Ins2Akita mice; however, COX-2 increases 2- and 2.75-fold in STZ and B6-Ins2Akita mice, respectively. Consistent with increased renal COX expression, PGE2 excretion is elevated in both STZ and B6-Ins2Akita mouse urine. We show a 1.9-fold increase in PGE2 in STZ mice (Fig. 3A), and both PGE2 and PGEM are increased 3.2- and 4.3-fold, respectively in B6-Ins2Akita mice (Fig. 3B). Similarly, in B6-Ins2Akita mice cortical COX receptors are increased 2.4-fold (Fig. 5A); however, the remaining EP receptor subtypes are significantly diminished 0.26-, 0.38-, and 0.47-fold for EP2, EP3, and EP4, respectively. In the medullary region of B6-Ins2Akita mice (Fig. 5B), both EP1 and EP3 are increased 5.5- and 1.95-fold, respectively, but EP2 and EP4 are unchanged.

**Table 1. Probes and primers used for real-time RT-PCR**

<table>
<thead>
<tr>
<th>Target</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
<th>Probe</th>
</tr>
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<tbody>
<tr>
<td>EP1 receptor</td>
<td>ccaagtgcagcagctggctcctgct</td>
<td>cccagtggaggtttgagtccttctgc</td>
<td>tggcctcgagggactgaagtccttctgc</td>
</tr>
<tr>
<td>EP3 receptor</td>
<td>gggccctgagtttgagtccttctgc</td>
<td>gccctggactgggtgtgcttctgc</td>
<td>ggcctggactgggtgtgcttctgc</td>
</tr>
<tr>
<td>EP4 receptor</td>
<td>tggcctcgagggactgaagtccttctgc</td>
<td>ggcctggaggtttgagtccttctgc</td>
<td>ggcctggaggtttgagtccttctgc</td>
</tr>
<tr>
<td>GAPDH</td>
<td>gggcctggactgggtgtgcttctgc</td>
<td>ggcctggaggtttgagtccttctgc</td>
<td>ggcctggaggtttgagtccttctgc</td>
</tr>
</tbody>
</table>

**Table 2. Summary of characteristics of 16-wk STZ-diabetic mice**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 3–4)</th>
<th>16-wk STZ-diabetic (n = 6–8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney wt, g (mean of both kidneys)</td>
<td>0.18±0.005</td>
<td>0.23±0.009</td>
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<tr>
<td>Body wt, g</td>
<td>37.1±1.2</td>
<td>26.7±1.4</td>
</tr>
<tr>
<td>Kidney/body wt</td>
<td>4.9±1.3</td>
<td>8.6±1.3</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>112±3</td>
<td>115±5</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>6.7±1.0</td>
<td>26.2±1.4†</td>
</tr>
<tr>
<td>Urine albumin/creatinine, ×10−1</td>
<td>6.5±1.0</td>
<td>4.1±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of mice; STZ, streptozotocin. *P < 0.05. †P < 0.001.

**Table 3. Summary of characteristics of 16-wk B6-Ins2Akita mice**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 4–8)</th>
<th>16-wk B6-Ins2Akita (n = 6–8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney wt, g (mean of both kidneys)</td>
<td>0.1475±0.0095</td>
<td>0.193±0.0127</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>25.4±2</td>
<td>23.7±0.3</td>
</tr>
<tr>
<td>Kidney/body wt</td>
<td>5.8±1.0</td>
<td>8.1±1.0</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>122.5±4.9</td>
<td>123.7±4</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>9.0±0.35</td>
<td>29.7±1.1†</td>
</tr>
<tr>
<td>Urine albumin/creatinine, ×10−1</td>
<td>2.95±0.69</td>
<td>11.29±1.18†</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of mice. *P < 0.05. †P < 0.001.

Fig. 1. Renal cyclooxygenase (COX) levels are altered in 16-wk streptozotocin (STZ)-induced diabetic mice. Protein was isolated from the cortex (A) and medulla (B) of control and 16-wk STZ-diabetic mice. COX-1 and -2 levels were determined by Western blotting. Densitometric analysis of COX is shown. Detection of β-actin was done to normalize samples. The ratio of COX to β-actin is presented as means ± SE (arbitrary units); n = 3–6. *P < 0.05.
**DISCUSSION**

Diabetes is a leading cause of chronic kidney disease (46). COX-derived PGs have been directly or indirectly implicated in diabetic kidney disease, initiating diabetic features or antagonizing other pathophysiological agents such as the renin-angiotensin system. EP1 receptor antagonists (29), prostacyclin receptor (IP) agonists (25, 37, 38), and thromboxane A2 synthase inhibitors (43) have proven to be beneficial, but the underlying mechanisms of PG involvement remain uncertain.

In the current study, we examined the levels of renal COX, urinary PGE2, and the expression of EP receptor subtypes in two recognized mouse models of type I diabetes, in the early stages of diabetic nephropathy before major changes in glomerular filtration rate (GFR). In both diabetic models, we observed an increase in the excretion of PGE2 as well as its metabolite in the B6-Ins2Akita mice. However, notable differences were observed in both models with respect to the expression of COX isoforms. While both COX-1 and COX-2 are increased in the cortex of STZ mice, only COX-1 increases in the cortex of B6-Ins2Akita mice. On the other hand, in the medulla, COX-1 is unchanged in the STZ mice and decreases in B6-Ins2Akita mice, but COX-2 increases in both models. Previously, we reported increased medullary levels of both COX isoforms in STZ-diabetic rats (35) as well as increased COX-2 levels in response to high glucose in rat mesangial cells (33, 34) and cultured inner medullary cells (35). Interestingly, in 4-wk STZ rats, Komers et al. (27) reported increased COX-2 and not COX-1 in the renal cortex and in a recent study found that only COX-2 is elevated in the obese Zucker fatty rat model of type II diabetes (26). The importance of elevated renal COX in diabetes is highlighted by the use of NSAIDS to inhibit COX.

**Table 4. Summary of real-time RT-PCR analysis of EP receptor expression in 16-wk control and diabetic mice**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STZ</td>
<td>Akita</td>
</tr>
<tr>
<td>EP1</td>
<td>Cortex</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td></td>
<td>Medulla</td>
<td>1.99±0.24</td>
</tr>
<tr>
<td>EP2</td>
<td>Cortex</td>
<td>2.04±0.49</td>
</tr>
<tr>
<td></td>
<td>Medulla</td>
<td>1.29±0.13</td>
</tr>
<tr>
<td>EP3</td>
<td>Cortex</td>
<td>1.1±0.13</td>
</tr>
<tr>
<td></td>
<td>Medulla</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>EP4</td>
<td>Cortex</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td></td>
<td>Medulla</td>
<td>0.81±0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE.
activity and PG synthesis. Only a few studies have reported the effects of NSAIDS on renal function in diabetes, but these are controversial due to differences in the type of NSAID studied and the duration of treatment (1, 10, 27, 31). Taken together, they indicate that COX-derived PGs contribute to diabetic alterations in the kidney and that the increase in COX-2 may play a significant role in the pathogenesis of diabetic kidney disease.

Another major finding in our current study is that there is altered EP receptor expression in diabetic mice kidneys, such that EP1 and/or EP3 receptors are increased in the renal cortex and/or medulla, but the EP4 and/or EP2 receptors are either decreased or unchanged. Of importance, however, are the obvious discrepancies in both diabetic models. For instance, in the renal cortex of STZ mice we showed increased EP1 and EP3 receptors and diminished EP4 receptors, whereas in B6-Ins2Akita mice, cortical EP1 receptors are increased, and the remaining EP receptor subtypes are significantly diminished. Similarly, in the medulla, in STZ mice EP1 receptors are diminished and EP3 receptors are increased, but in B6-Ins2Akita mice both EP1 and EP3 are increased. Whether these differences are attributable to actual diversity in the diabetic state of the mice, or are related to the use of STZ, remains to be determined. Nonetheless, a disturbance in EP-mediated responses could surely influence the course of diabetic kidney disease. Since we have shown that renal PGE2 is elevated, this could have an impact on glomerular and collecting duct function, two major sites of renal PGE2 synthesis and action. For instance, within the kidney, a majority of EP1 mRNA is found in the collecting duct (8), and in the present study we show the largest increase in EP1 receptor mRNA relative to other EP receptors. Defective PGE2/EP receptor signaling could interfere with the fine-tuning of salt and water transport, and these abnormalities could contribute to edema, hypertension, and vascular changes associated with diabetic nephropathy. To further support this idea, the significance of PGE2 to the maintenance of salt and water homeostasis is clearly demonstrated by the undesirable renal effects such as sodium and potassium retention (39) associated with the use of NSAIDS, which inhibit the production of PGs. Therefore the increase in EP1 receptors reported in this paper would surely contribute to renal salt and H2O alterations seen in diabetes. Future studies in our laboratory will target EP1 receptors with pharmacological or molecular interventions at various stages of diabetic disease.

Fig. 4. Renal EP mRNA expression is altered in 16-wk STZ-diabetic mice. Real-time RT-PCR detection of EP1-4 receptor mRNA was performed on total RNA samples isolated from the cortex (A) and medulla (B) of control and 16-wk STZ-diabetic mice using specific probes and primers for each EP receptor subtype. GAPDH mRNA was detected as an internal control. The ratio of EP mRNA to GAPDH mRNA is presented as means ± SE (n = 3–5) expressed as fold-control (control = 1). *P < 0.05.

Fig. 5. Renal EP mRNA expression is altered in 16-wk B6-Ins2Akita mice. Real-time RT-PCR detection of EP1-4 receptor mRNA was performed on total RNA samples isolated from the cortex (A) and medulla (B) of control and 16-wk B6-Ins2Akita diabetic mice using specific probes and primers for each EP receptor subtype. GAPDH mRNA was detected as an internal control. The ratio of EP mRNA to GAPDH mRNA is presented as means ± SE (n = 3–5) expressed as fold-control (control = 1). *P < 0.05.
nephropathy to clarify the role of EP1 in the development of diabetic complications.

In our study, we also observed an increase in EP3 receptors, which could play a part in diabetic changes in the kidney. A similar deregulation of cortical EP3 responses is proposed to play a role in the progression of kidney disease in rats with passive Heymann nephritis (45). Our group and others have demonstrated an important role for PGE2 in the collecting duct to limit AVP responses (17–19); thus the elevated EP3 receptor levels reported here could serve to oppose AVP-mediated H2O reabsorption and reduce volume expansion in diabetes (2).

The highest intrarenal expression of the EP1 receptor is reported in the cortex (8, 21, 42). In contrast, in our study we show higher expression of EP4 in the renal medulla. Furthermore, our work indicates that EP4 receptor mRNA is either diminished or unchanged in diabetes, which could also affect the progression of diabetic change in the tubule and glomeruli. For example, it is clear that PGE2 activates EP4 receptors located on collecting duct principal cells to stimulate H2O reabsorption via aquaporin-2 (24, 44). Therefore, the significance of diminished EP4 receptors in our study could be to prevent excessive H2O reabsorption in the diabetic collecting duct that would otherwise lead to volume expansion and further supports the diuretic role of PGE2 in diabetes. Additionally, a defect in EP3 receptors could influence glomerular function. For example, in podocytes EP4 receptors are important in preventing the morphological changes required for podocytes to adapt to mechanical stretch in vitro, which could contribute to proteinuria in hypertensive patients, for example (30). Also, our group and others showed that in mesangial cells cAMP-stimulating PGs alter cell proliferation and matrix turnover (20, 33, 34), thus contributing to diabetic glomerular disease. Since EP2 receptors are mainly found in the renal vasculature and interstitial cells, the reduction in cortical EP2 receptors found in our study could influence vascular and interstitial cell function in diabetes.

It is noteworthy to recognize the discrepancies between the two diabetic models presented in this paper considering the known toxicity of STZ and that B6-Ins2Akita mice develop more severe diabetic complications in the kidney (14). Interestingly, we did not detect any changes in blood pressure in both models, and a study by Kakoki et al. (22) indicates that blood pressure in 6-mo B6-Ins2Akita mice is not significantly different from that in controls. In contrast, a recent study by Gurley et al. (15) reports a significant increase in systolic blood pressure in 16-wk B6-Ins2Akita mice. The reason for this discrepancy is not clear at this time.

Also, the alterations in the EP receptor expression profile in these mice are noted before any major disturbances in renal function or structural changes due to diabetes, although the kidneys are enlarged in both models relative to body weight, and in the B6-Ins2Akita mice we did observe an increase in urine albumin. Our work suggests that the defect in EP1 (2 +/or 4) receptors relative to EP1 (1 +/or 3) pathways reported here may be important in the initiation of diabetic change, before changes in GFR. It is also interesting that there is a similar disturbance in the ratio of (PGE2 and PGJ2) to thromboxane A2 in diabetes that has been implicated in the progressive loss of renal function and development of diabetic nephropathy (9, 11–13). Together, a defect in PGE2/EP1 (2 +/or 4) responses relative to PGE2/EP1 (1 +/or 3) responses will surely serve to perpetuate the diabetic complications in the kidney, but further staging of the changes in EP receptor expression as diabetic characteristics arise will be needed to confirm the role of each subtype in the progression of diabetic kidney disease.

In summary, our work shows that renal COX levels and urinary PGE2 excretion are increased in both STZ-diabetic and B6-Ins2Akita mice, two models of type 1 diabetes, before major diabetic changes in renal function. There is an altered expression profile of EP receptors throughout the diabetic kidney, mainly favoring EP1/3 receptor-mediated responses, suggesting a role in the onset of diabetic change. Further studies will clarify the significance of these findings to disturbances in glomerular and collecting duct function and progression of diabetic nephropathy by stage the disturbances in EP receptor expression as diabetic features occur in B6-Ins2Akita mouse kidneys. The use of EP receptor knockout technology as well as specific agonists and antagonists should facilitate these future endeavors. Once clarified, this could lead to the advent of better combination therapy to prevent the progression of the disease or reverse diabetic complications (16–19).

GRANTS

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