

Intrarenal Dopamine Inhibits Progression of Diabetic Nephropathy

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The kidney has a local intrarenal dopaminergic system, and in the kidney, dopamine modulates renal hemodynamics, inhibits salt and fluid reabsorption, antagonizes the renin-angiotensin system, and inhibits oxidative stress. The current study examined the effects of alterations in the intrarenal dopaminergic system on kidney structure and function in models of type 1 diabetes. We studied catechol-*O*-methyl-transferase (COMT)^{-/-} mice, which have increased renal dopamine production due to decreased dopamine metabolism, and renal transplantation was used to determine whether the effects seen with COMT deficiency were kidney-specific. To determine the effects of selective inhibition of intrarenal dopamine production, we used mice with proximal tubule deletion of aromatic amino acid decarboxylase (ptAADC^{-/-}). Compared with wild-type diabetic mice, COMT^{-/-} mice had decreased hyperfiltration, decreased macula densa cyclooxygenase-2 expression, decreased albuminuria, decreased glomerulopathy, and inhibition of expression of markers of inflammation, oxidative stress, and fibrosis. These differences were also seen in diabetic mice with a transplanted kidney from COMT^{-/-} mice. In contrast, diabetic ptAADC^{-/-} mice had increased nephropathy. Our study demonstrates an important role of the intrarenal dopaminergic system to modulate the development and progression of diabetic kidney injury and indicate that the decreased renal dopamine production may have important consequences in the underlying pathogenesis of diabetic nephropathy. *Diabetes* 61:2575–2584, 2012

Diabetic nephropathy is the most prevalent cause of end-stage kidney disease in the U.S. It is characterized by hemodynamic alterations and progressive glomerular and tubulointerstitial injury. Although our understanding of the underlying pathophysiologic mechanisms has advanced remarkably in the past few years, the factors that promote or retard the development and progression of diabetic renal injury remain incompletely understood.

It is now well-known that the renin-angiotensin system (RAS) is an important mediator of abnormal renal function and structure in diabetic nephropathy, and RAS inhibition represents an important therapeutic target clinically. In

addition, renal cyclooxygenase-2 (COX-2) expression also increases in experimental models of diabetes (1–3) as well as in human diabetic nephropathy (4), and studies in animals have indicated that COX-2 inhibition can inhibit both renal hemodynamic and structural abnormalities of diabetic nephropathy (1–3).

Dopamine mediates important physiologic functions in the mammalian kidney. It modulates renal hemodynamics and regulates salt and water reabsorption by inhibiting both proximal and distal solute and water transport, effects mediated by inhibition of specific tubule transporter activity (5). The kidney serves as a major source of dopamine production separate from any neural production. Circulating dopamine is usually in the picomolar range, whereas dopamine levels in the kidney can reach high nanomolar concentrations (6). The dopamine precursor L-dihydroxyphenylalanine (L-DOPA) is taken up in the proximal tubule from the circulation or following filtration at the glomerulus and is then converted to dopamine in the proximal tubule by aromatic amino acid decarboxylase (AADC). In the kidney, dopamine is metabolized predominantly by catechol-*O*-methyl-transferase (COMT), with a smaller contribution by monoamine oxidase A.

In addition to its direct diuretic and hemodynamic effects, there is also evidence that dopamine can modulate angiotensin signaling and can inhibit oxidant stress in the kidney (7–9). We have also previously documented interactions between the intrarenal dopaminergic and COX-2 systems (10–13). Renal cortical COX-2 expression is inversely related to intravascular volume and is a mediator of juxtaglomerular renin expression (14). Increased proximal tubule dopaminergic activity inhibits COX-2 expression in the macula densa (12).

Previous studies have suggested that abnormalities in the intrarenal dopaminergic system may be an underlying contributing factor to increased renal salt and water reabsorption in diabetes (15,16). Diabetes is characterized by elevated glomerular filtration rate (GFR) and sodium retention due to increased proximal reabsorption and reduced luminal NaCl delivery to the macula densa (17,18). An impaired intrarenal dopaminergic system in diabetes may contribute to reduced luminal NaCl delivery at the macula densa, resulting in the elevated macula densa COX-2 expression seen in experimental diabetes and diabetic patients. In addition, impairments in the intrarenal dopaminergic system may lead to increased RAS-dependent renal injury and increased inflammatory responses. Therefore, the current studies examined the potential role of the intrarenal dopaminergic system to modulate progression of diabetic nephropathy in mouse models of type 1 diabetes.

RESEARCH DESIGN AND METHODS

Animals. COMT^{-/-} and wild-type mice on the 129J/sv background were obtained from Dr. Maria Karayiorgou at Rockefeller University (19). COMT^{-/-}

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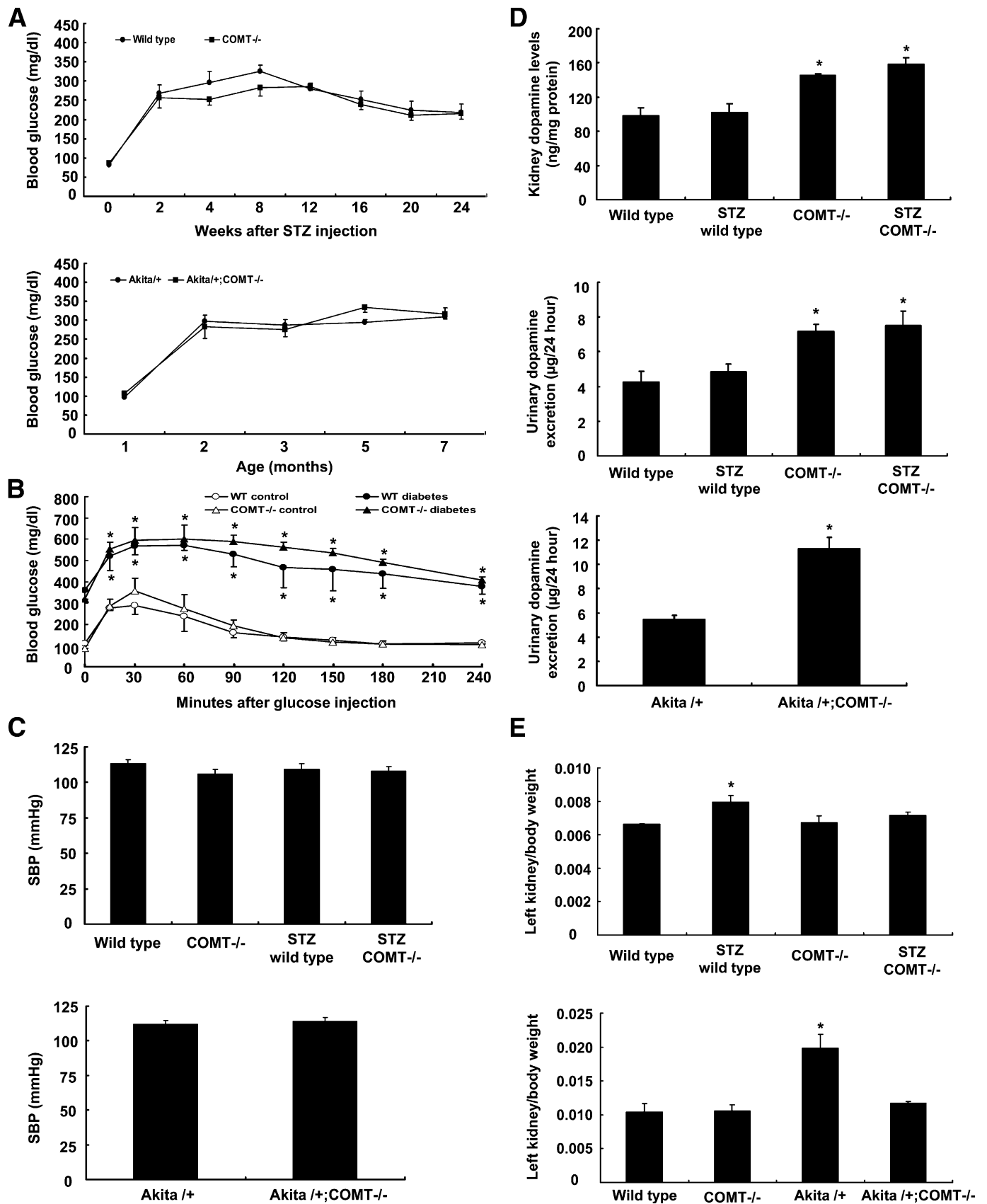


FIG. 1. Hyperglycemia and systolic blood pressure (SBP) were equivalent in wild-type and COMT^{-/-} mice with type 1 diabetes. **A:** Blood sugars were equivalent in both STZ (top) and Akita⁺ (bottom panel) models ($n = 8$). **B:** There was no difference in glucose tolerance in the STZ model in wild-type and COMT^{-/-} mice ($n = 4$). $*P < 0.05$. **C:** Tail cuff blood pressures were not different in either diabetic model ($n = 6$). **D:** Kidney (top panel) and urinary (middle and bottom panels) dopamine levels were increased in COMT^{-/-} mice with or without diabetes. $*P < 0.01$; $n = 4$. **E:** Kidney hypertrophy was inhibited in COMT^{-/-} diabetic mice compared with wild-type mice. $*P < 0.05$, $n = 4$.

mice were crossed with Akita⁺ mice on the 129J/sv background. As previously reported (13), *Aadc^{fllox7/fllox7}* mice were crossed with mice in which Cre is under the control of the γ -GT promoter (proximal tubule AADC gene deletion [*ptAADC^{-/-}*] mice) and backcrossed for 10 generations onto the 129/svj background. The institutional animal care and use committee of Vanderbilt University approved all protocols. At 2 months of age, male mice received daily injections for 5 consecutive days of streptozotocin (STZ; 50 mg/kg i.p.) that was freshly prepared in 0.1 mol/L citrate buffer (pH 4.5). The onset of diabetes was evaluated by measuring fasting blood glucose. Blood glucose was measured using a B-glucose analyzer (HemoCue, Lake Forest, CA) in conscious mice on saphenous vein samples at noon after a 6-h fast.

Glucose tolerance test. After fasting overnight (16 h), the mice were injected intraperitoneally (i.p.) with glucose at a dose of 2 g/kg. Blood glucose was monitored at different time points after glucose administration.

Twenty-four-hour urine collection and measurement of urinary albumin excretion. Mice were trained three times in metabolic cages (Braintree Scientific, Braintree, MA) before 24-h urine collections. Briefly, a single mouse was put into a metabolic cage for 24 h and then returned to its original cage for 2 days before the next training period. The metabolic cages were moisturized to minimize the evaporation of urine sample when 24-h urines were collected. Urine albumin concentrations were determined by using a commercially available kit (Exocell).

Measurement of GFR in conscious mice. GFR in conscious mice was measured as we have reported previously (20).

Measurement of urine and kidney dopamine. Dopamine levels in the kidney and urine samples were measured by high-performance liquid chromatography in the Neurochemistry Core Laboratory at Vanderbilt University's Center for Molecular Neuroscience Research (21).

Determination of urinary F₂-isoprostane. Prostaglandin F₂-like compounds, the F₂-isoprostanes have been recognized as sensitive and specific biomarkers for oxidative stress (22). Urinary F₂-isoprostane levels were determined by negative-ion gas-chromatography mass spectrometry (23).

Immunohistochemistry. Animals were anesthetized with Nembutal (70 mg/kg i.p.), given heparin (1,000 units/kg i.p.) to minimize coagulation, and perfused with 3.7% formaldehyde, 10 mmol/L sodium *m*-periodate, 40 mmol/L phosphate buffer, and 1% acetic acid through the aortic trunk cannulated by means of the left ventricle. The 3.7% formaldehyde, 10 mmol/L sodium *m*-periodate, 40 mmol/L phosphate buffer, and 1% acetic acid is an acidified aldehyde, which provides excellent preservation of tissue structure, antigenicity, and mRNA (24). The fixed kidney was dehydrated through a graded series of ethanols, embedded in paraffin, sectioned (4 μ m), and mounted on glass slides. Immunohistochemical staining was carried out as in previous reports (13). The primary antibodies that were used for immunohistochemical studies included rabbit polyclonal antimurine COX-2 antibody (160106; Cayman Chemicals), rabbit polyclonal anti-human fibronectin antibody (F3648; Sigma-Aldrich), goat polyclonal anti-human connective tissue growth factor (CTGF) antibody (SC-14939; Santa Cruz Biotechnology), rabbit antimurine collagen type I antibody (AB765P; Millipore), rabbit anti-fibroblast-specific protein-1 (FSP-1) antibody (gift from Dr. E.G. Neilson), rabbit antimurine collagen type IV antibody (T40263R; Biodesign International), affinity-purified rabbit anti-AADC antibody (AB136; Chemicon International), rat anti-mouse F4/80 (marker of macrophages, MCA497R; AbD Serotec), and rabbit antinitrotyrosine (marker of oxidative stress, SC-55256; Santa Cruz Biotechnology).

Immunoblotting. Kidney samples were homogenized with radioimmuno-precipitation assay buffer and centrifuged, and an aliquot was taken for protein measurement. When Western blot analysis was performed, each lane was loaded with the same amount of protein. The proteins were separated on SDS-PAGE under reducing conditions and transferred to Immobilon-P transfer membranes (Millipore). After blocking with 20 mmol/L Tris/HCl (pH 7.4)/500 mmol/L NaCl/5% nonfat milk/0.1% Tween 20 for 3 h at room temperature, the blots were incubated overnight at 4°C with rabbit polyclonal antimurine COX-2 antibody (1:2,000), rabbit polyclonal anti-human fibronectin antibody (1:6,000), rabbit anti-FSP-1 antibody (1:1,000), and goat polyclonal anti-human CTGF antibody (1:100). The primary antibodies were detected with peroxidase-labeled goat anti-rabbit IgG or donkey anti-goat IgG (Santa Cruz

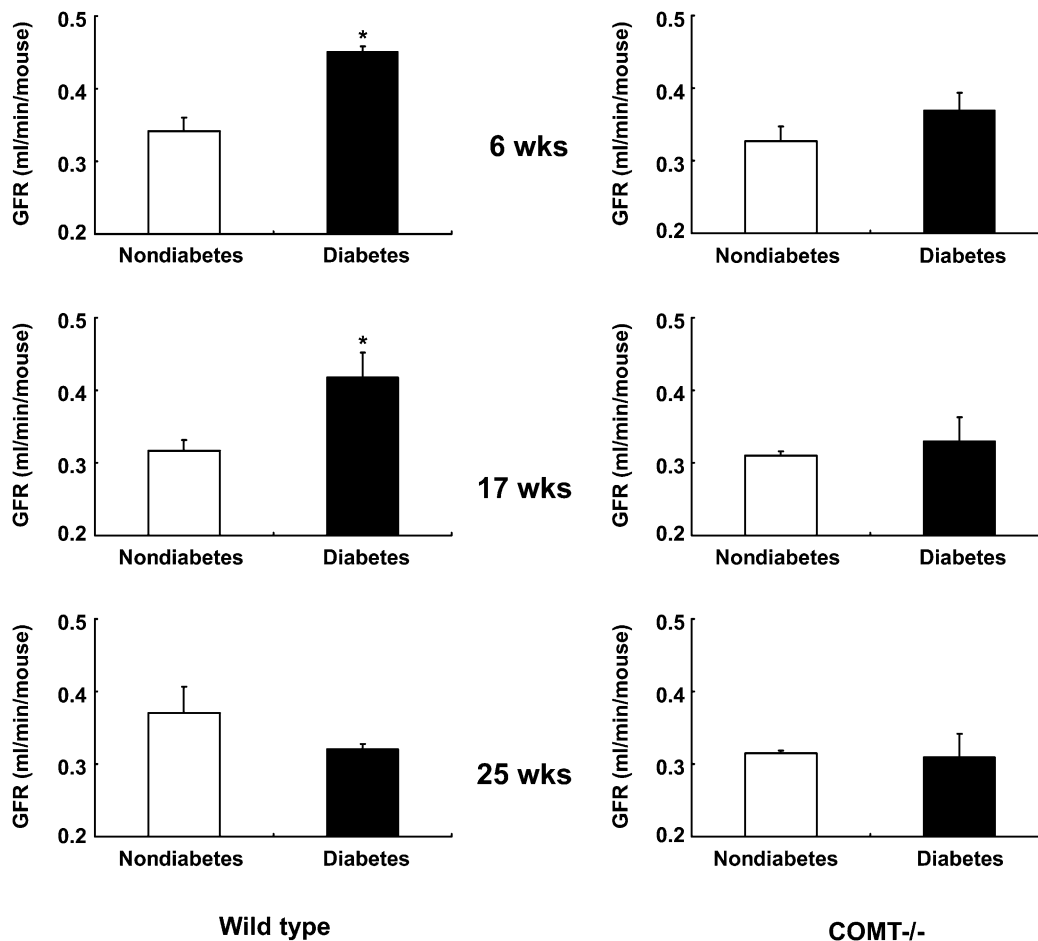


FIG. 2. Compared with nondiabetic mice, wild-type STZ diabetic mice had increased GFR, measured by fluorescein isothiocyanate inulin, at 6 (top panels) and 17 (middle panels) weeks (wks) after induction of diabetes, whereas there were no statistically significant increases in GFR in COMT^{-/-} mice. **P* < 0.05; *n* = 4.

Biotechnology) and exposed on film by using enhanced chemiluminescence (Amersham International).

Renal histopathology. The kidney sections were stained with periodic acid Schiff for light microscopic examination. For electron microscopy, kidney cortex samples were fixed in 2.5% glutaraldehyde in 0.1 mmol/L cacodylate buffer (pH 7.4). After fixation, samples were dehydrated through a graded series of ethanol and embedded in Spurr's resin. The sections (80–100 nm) were viewed using an FEI/Philips CM12 transmission electron microscope (Philips).

Kidney transplantation. Kidney transplantation was performed on male mice weighting 20–30 g. Mice were anesthetized with ketamine (80 mg/kg i.p.) and xylazine (5 mg/kg i.p.) mixed in a normal saline solution. Via a midline incision, the renal vein and artery were dissected apart, the kidney was then separated from the perinephric fat and the adrenal gland, and then the ureter was dissected freely down to the bladder and cut with a small (1 to 2 mm) bladder patch. After clamping the aorta above the renal artery, a 30-gauge needle was introduced into the aorta, the inferior (caudal) vena cava was cut and the graft perfused with 1.0 mL cold heparinized saline solution (100 U/mL). Renal vessels were cut with a Carrel patch of the aorta and vena cava, and the graft was removed and stored in a normal saline solution at 4°C for 20 min until the time of transplantation. For the recipient, a midline incision from the xyphoid appendix to pubis was made and exposure achieved using small retractors to keep liver and bowels away from the kidney. The renal pedicle was ligated with 7–0 silk sutures, and the

recipient's left kidney was removed, leaving space for the donor kidney. After ligating some lumbar branches, the infrarenal aorta and inferior vena cava were isolated, and two loops of 7–0 silk were placed proximally and distally around them and tied to promote homeostasis. Elliptical longitudinal aortotomy and cavotomy were performed between these ties, and the vessels were flushed to clear blood inside them. Vascular anastomoses were performed end-to-side to the abdominal aorta and vena cava using a running 11–0 nylon suture (Ethilon; Ethicon). Urinary reconstruction was performed by bladder-to-bladder anastomosis via a large bladder patch. Abdominal wall and skin were closed with a continuous 5–0 absorbable suture (Vycryl; Ethicon). One week later, the right kidney of the recipient mouse was removed via a dorsal approach.

Statistics. All data are presented as means \pm SE. ANOVA and *t* tests were used for data analysis. A *P* value <0.05 was considered as significant.

RESULTS

We have previously reported that $COMT^{-/-}$ mice have increased renal and urinary dopamine levels (12). To investigate the potential role of the intrarenal dopaminergic system in diabetic nephropathy, we studied male $COMT^{-/-}$ mice and wild-type mice on the 129J/sv background. Type 1 diabetes was induced either by low-dose STZ injections (25) or by crossing with mice with the same genetic background that were heterozygous for the Akita mutation on the same background (26,27). With either maneuver to induce diabetes, blood glucose elevations were similar in wild-type and $COMT^{-/-}$ mice (Fig. 1A). As further confirmation, we determined that the glycemic responses in glucose tolerance tests were similar between control or diabetic wild-type and $COMT^{-/-}$ mice (Fig. 1B). Systolic blood pressures measured by tail cuff sphygmomanometry were also not different between diabetic wild-type and $COMT^{-/-}$ mice (Fig. 1C). In both the STZ and Akita^{+/+} models of diabetes, renal dopamine levels and urinary dopamine excretion were significantly greater (Fig. 1D), and renal hypertrophy was significantly less (Fig. 1E) in $COMT^{-/-}$ diabetic mice than in wild-type diabetic mice. Our results are in agreement with other reports that pharmacologic inhibition of COMT activity alone leads to increased intrarenal dopaminergic activity (28–30).

GFR was significantly higher in wild-type mice with STZ-induced diabetes compared with nondiabetic mice at 6 and 17 weeks, whereas by 25 weeks, there was numerically but not significantly decreased GFR. In contrast, there was no evidence of hyperfiltration in the diabetic $COMT^{-/-}$ mice at any time point studied (Fig. 2). Previous studies have shown that macula densa COX-2 expression increases in hyperfiltering states (2,31,32), including early diabetes. We have also shown previously that dopamine can modulate macula densa COX-2 expression. Macula densa COX-2 expression increased within two weeks in wild-type diabetic mice, but the increased expression was significantly blunted in $COMT^{-/-}$ diabetic mice (Fig. 3A and B). Acute administration of the selective COX-2 inhibitor SC58236 (2 mg/kg, gastric gavage, given 1 h before GFR assay) decreased the elevated GFR in wild-type diabetic mice at 6 weeks back to levels seen in nondiabetic control animals (0.345 ± 0.016 vs. 0.451 ± 0.019 mL/min/mouse; $n = 6$, $P < 0.05$). In contrast, COX-2 inhibition did not significantly decrease GFR in diabetic $COMT^{-/-}$ mice (Fig. 3C).

Significant albuminuria was observed in both the STZ and Akita^{+/+} models of diabetes in wild-type mice (Fig. 4A). In contrast, albuminuria was significantly less in diabetic $COMT^{-/-}$ mice (Fig. 4A). In diabetic $COMT^{-/-}$ mice, there was also decreased mesangial expansion (Fig. 4B) and decreased glomerular basement membrane (GBM) thickening and foot process effacement (Fig. 4C) compared

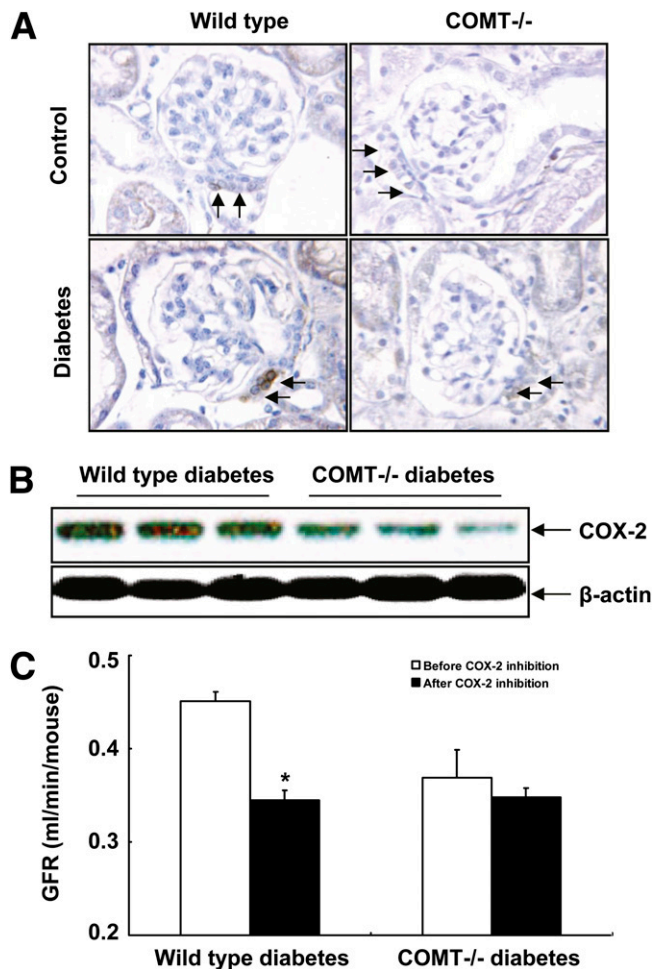


FIG. 3. Alterations in macula densa COX-2 expression in diabetes. **A:** Immunolocalization of macula densa COX-2 (arrows) indicated increased expression in wild-type STZ diabetic mice, with significant blunting in $COMT^{-/-}$ diabetic mice. **B:** Immunoblotting of renal cortex from wild-type and $COMT^{-/-}$ STZ diabetic mice. **C:** Increases in GFR seen in wild-type mice 6 weeks after induction of STZ-induced diabetes were significantly blunted by the COX-2 selective inhibitor SC58236, whereas it had no significant effect in $COMT^{-/-}$ diabetic mice. * $P < 0.05$; $n = 4$. (A high-quality color representation of this figure is available in the online issue.)

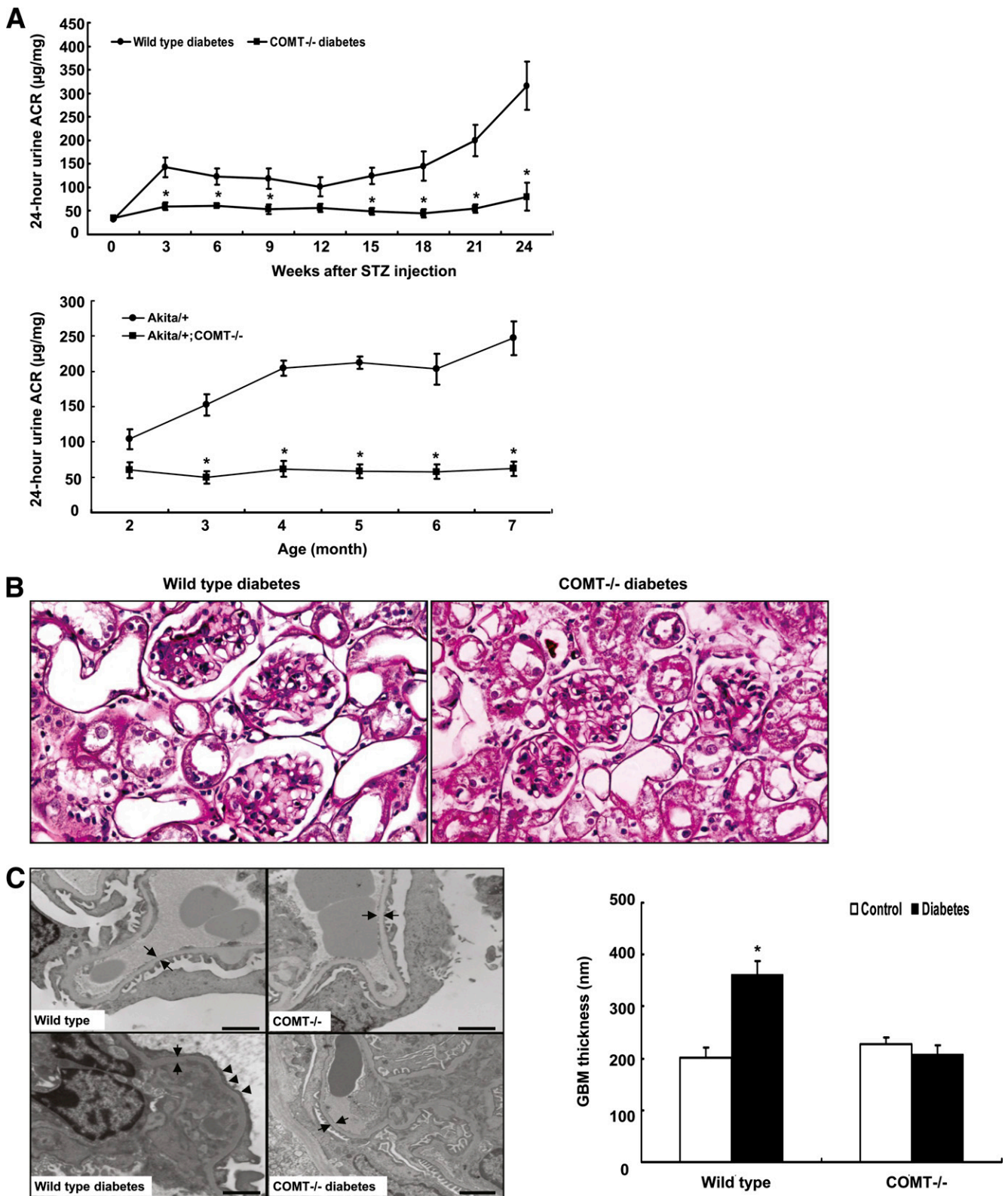


FIG. 4. A: Albuminuria, measured by 24-h urinary albumin/creatinine ratios (ACR), increased significantly in both STZ (*top panel*) and Akita⁺ (*bottom panel*) wild-type diabetic mice compared with $\text{COMT}^{-/-}$ diabetic mice. * $P < 0.05$; $n = 6-8$. **B:** There was increased mesangial expansion noted in wild-type diabetic mice compared with $\text{COMT}^{-/-}$ diabetic mice 24 weeks after induction of STZ-induced diabetes (periodic acid Schiff; $\times 400$ original magnification). **C:** Electron microscopy indicated increased foot process effacement (*arrowheads*) and statistically significant increases in GBM thickness (*arrows and bar graph*) in wild-type diabetic mice compared with $\text{COMT}^{-/-}$ diabetic mice. * $P < 0.01$; $n = 3$. **D:** Immunoblotting and immunohistochemistry indicated increased expression of CTGF, fibronectin, FSP-1, collagen I, and collagen IV in wild-type diabetic mice compared with $\text{COMT}^{-/-}$ diabetic mice. (A high-quality digital representation of this figure is available in the online issue.)

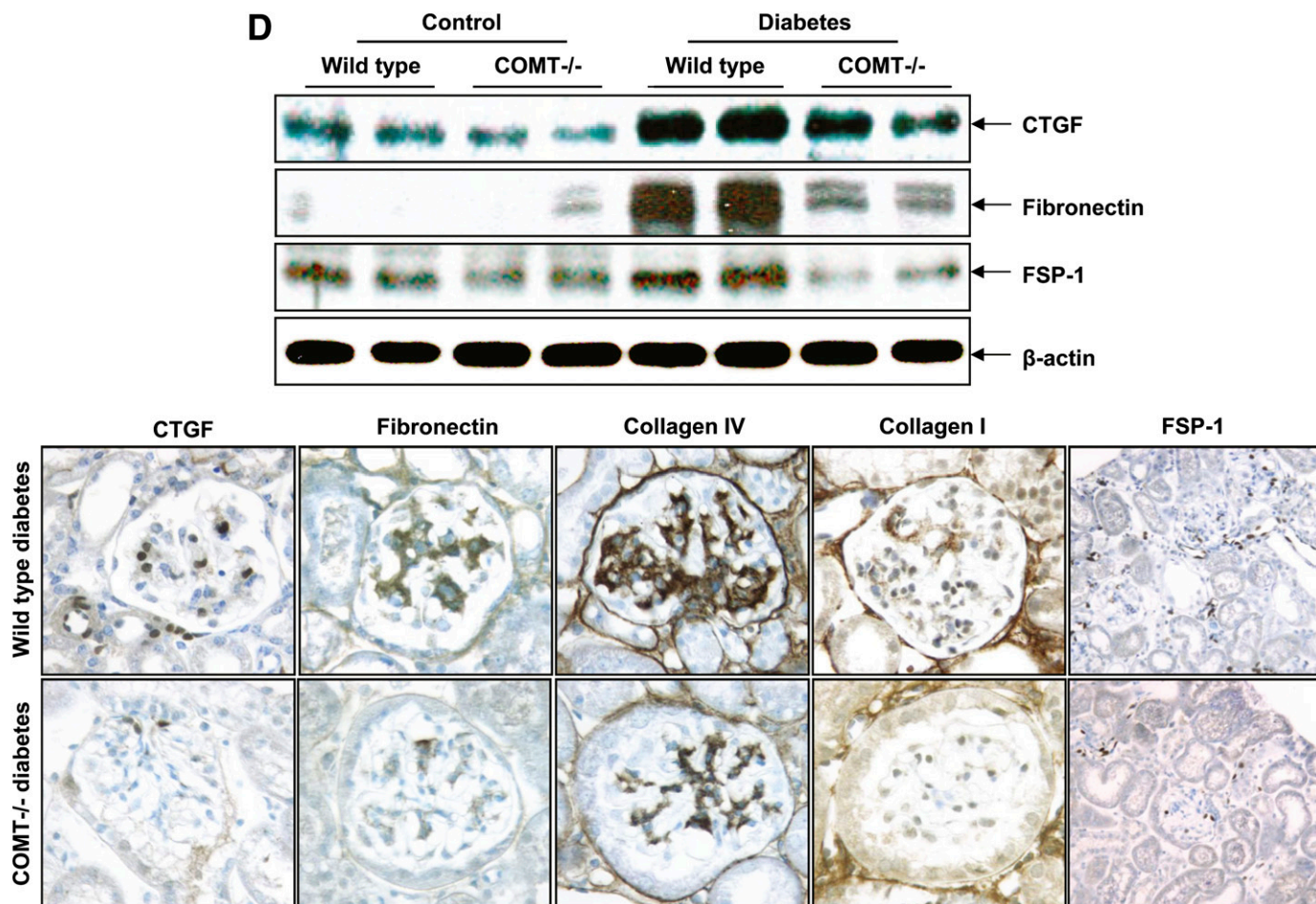


FIG. 4. Continued.

with wild-type diabetic mice. In wild-type diabetic mice, expression of both CTGF and the fibroblast marker FSP-1 was increased in both glomerular and interstitial compartments, as well as expression of fibronectin and type I and IV collagens, but the increases in expression of these components of fibrosis were inhibited in COMT^{-/-} mice (Fig. 4D). It should be noted that kidney expression of CTGF and fibronectin was only partially inhibited in diabetic COMT^{-/-} mice, it was still higher compared with that in control COMT^{-/-} mice (Fig. 4D). In addition, diabetic COMT^{-/-} mice had less macrophage infiltration (Fig. 5A) as well as decreased markers of oxidative stress, nitrotyrosine (Fig. 5A), and urinary F₂-isoprostane excretion (Fig. 5B).

COMT^{-/-} mice have global deletion of the COMT gene. In order to determine whether the observed protective effects against development of diabetic nephropathy were due entirely to increased intrarenal dopamine, we transplanted kidneys from either wild-type or COMT^{-/-} mice into bilaterally nephrectomized wild-type mice. Unilaterally nephrectomized wild-type mice were used as controls. Diabetes was induced by STZ in all three groups of mice. Urinary dopamine excretion was markedly higher in diabetic mice with a transplanted COMT^{-/-} kidney than in diabetic mice with a transplanted wild-type kidney (3.56 ± 0.68 vs. 1.38 ± 0.38 $\mu\text{g}/24$ h; $n = 5$, $P < 0.05$). As indicated in Fig. 6A, albuminuria increased comparably in uninephrectomized mice and in mice transplanted with a wild-type

kidney. In contrast, the increases in albuminuria were significantly blunted in diabetic mice with a transplanted COMT^{-/-} kidney. There was also decreased mesangial expansion, macrophage infiltration, and nitrotyrosine expression (Fig. 6B), as well as less tubulointerstitial fibrosis (Fig. 6C).

We have previously described a model of selective intrarenal dopamine deficiency in which mice with a floxed AADC gene were crossed with γ -GT Cre mice, resulting in selective ptAADC^{-/-} and marked decreases in renal and urinary dopamine levels (13). Diabetes was induced with STZ in the ptAADC^{-/-} mice and wild-type littermates. Blood glucose levels were similar in the two groups (Fig. 7A). However, in ptAADC^{-/-} mice, albuminuria was markedly increased (Fig. 7B). Compared with wild-type littermates, these diabetic mice also had markedly increased mesangial expansion, increased renal macrophage infiltration, and increased nitrotyrosine staining (Fig. 7C).

DISCUSSION

The current studies demonstrate that intrarenal dopamine serves as an important modulator of diabetic kidney injury. Mice with selective intrarenal deficiency of AADC, the enzyme responsible for dopamine production from its precursor, L-DOPA, had increased albuminuria and worsened structural renal damage in a model of type 1 diabetes. Conversely, in COMT^{-/-} mice, in which intrarenal

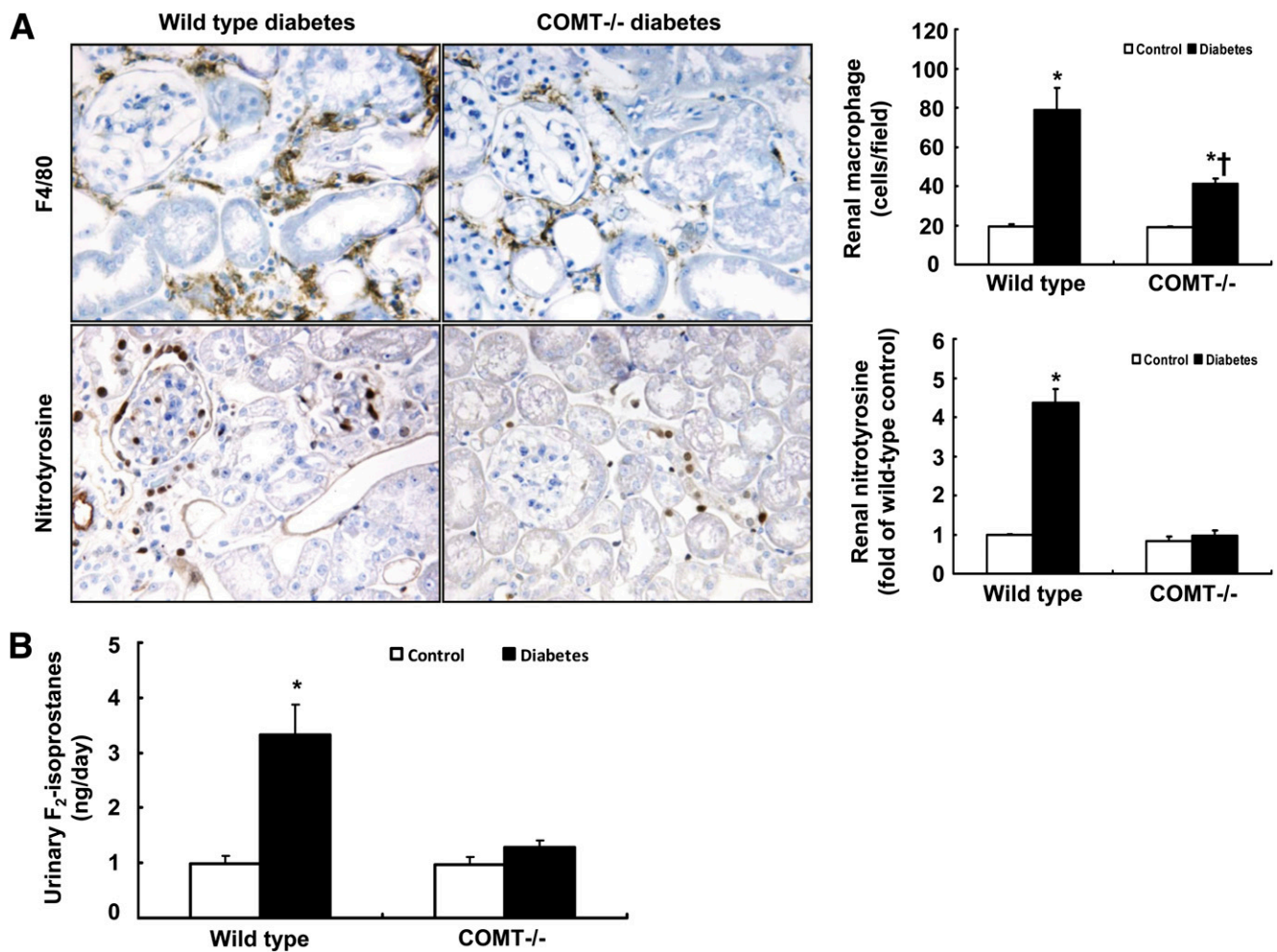


FIG. 5. Increases in markers of inflammation and oxidative stress. **A:** There was increased macrophage infiltration (indicated by F4/80 immunorexpression) and increased nitrotyrosine staining noted in wild-type diabetic mice compared with COMT^{-/-} diabetic mice 24 weeks after induction of STZ-induced diabetes. * $P < 0.01$ compared with diabetes, † $P < 0.05$ compared with wild-type diabetes; $n = 4$. **B:** Urinary isoprostane excretion. * $P < 0.01$ compared with no diabetes; $n = 4$. (A high-quality color representation of this figure is available in the online issue.)

dopamine metabolism to inactive metabolites is inhibited, there was a decrease in albuminuria and histological abnormalities. That this effect was mediated specifically by intrarenal dopamine was confirmed by the demonstration that kidneys transplanted from COMT^{-/-} mice into wild-type mice had markedly less severe diabetic nephropathy than mice with transplanted wild-type kidneys.

Previous experimental and clinical studies have identified a range of potential complementary mechanisms underlying the development of diabetic nephropathy (33), including toxic effects of elevated glucose and/or advanced glycosylation end products, hemodynamic alterations, oxidative stress, inflammation, and local activation of the RAS. Although blood glucose was not different between diabetic wild-type mice and mice with either increased or decreased intrarenal dopamine levels, intrarenal dopamine modulated other potential mediators of diabetic nephropathy. Increased intrarenal dopamine levels inhibited hyperfiltration, decreased markers of oxidative stress, and inhibited macrophage infiltration, whereas decreased intrarenal dopamine production had the opposite effect.

Defective autoregulation of renal blood flow due to decreased myogenic tone of the afferent arteriole and

resetting of tubuloglomerular feedback to a higher distal tubular flow rate underlies hyperfiltering states and is corrected by inhibition of COX activity (34). Macula densa COX-2 expression increases in models of hyperfiltration, such as renal ablation, high-protein diet, and diabetes, and treatments that inhibit COX-2 decrease hyperfiltration (2,31,35). Our previous studies indicated that intrarenal dopamine can inhibit increases in macula densa COX-2 expression in response to alterations in volume status (10–13). The current studies demonstrate that increased intrarenal dopamine would also decrease the increased macula densa COX-2 expression seen in diabetes. That this inhibition in COX-2 expression underlies the lack of hyperfiltration seen in the COMT^{-/-} mice was confirmed by the observation that a selective COX-2 inhibitor blocked hyperfiltration in wild-type diabetic mice but did not affect GFR in diabetic COMT^{-/-} mice. The current results are also in agreement with, and provide a mechanistic explanation for, earlier studies that showed administration of L-DOPA or the DA1 agonist fenoldopam decreased hyperfiltration in diabetic rats (36,37).

Increased intrarenal dopamine also blunted increases in mesangial matrix and GBM thickening, as well as inhibiting expression of mediators (CTGF) and markers of

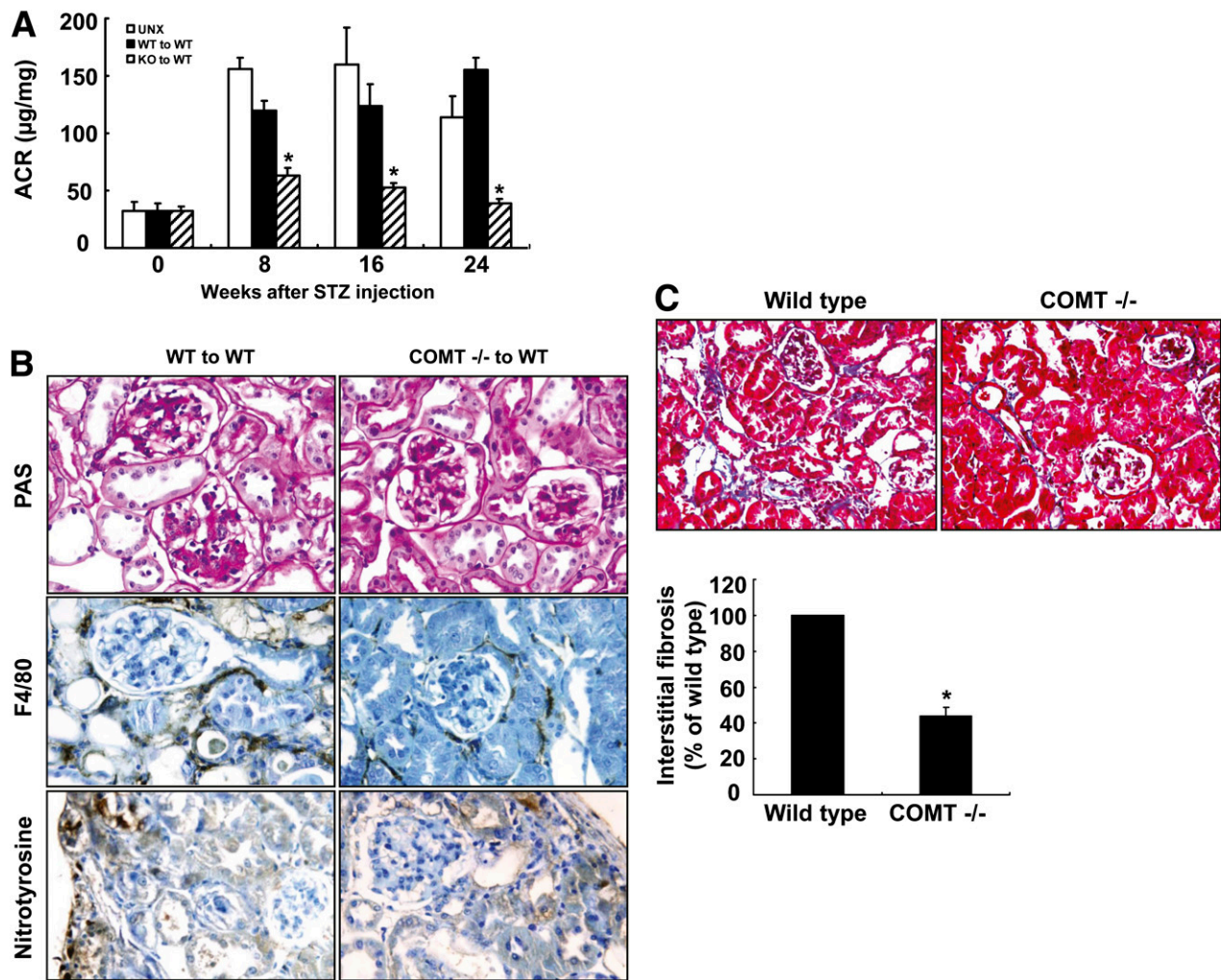


FIG. 6. Diabetic nephropathy in transplanted kidneys. **A:** Compared with wild-type (WT) mice with unilateral nephrectomy or bilateral nephrectomy and transplantation of a wild-type kidney, bilaterally nephrectomized wild-type mice with transplantation of a COMT^{-/-} kidney had significantly decreased albumin/creatinine ratios (ACR) in response to STZ-induced diabetes. * $P < 0.05$; $n = 4$. UNX, uninephrectomized. **B:** The diabetic mice with a COMT^{-/-} transplanted kidney had less mesangial expansion, macrophage infiltration, and nitrotyrosine staining ($\times 400$ original magnification). PAS, periodic acid Schiff. **C:** Mice with a COMT^{-/-} transplanted kidney had decreased tubulointerstitial fibrosis, as indicated by Masson Trichrome staining ($\times 400$ original magnification). * $P < 0.05$; $n = 4$. (A high-quality digital representation of this figure is available in the online issue.)

fibrosis (collagen I, collagen IV, fibronectin, and FSP-1). Of note, previous studies have indicated that COX-2 inhibition not only decreased hyperfiltration in the diabetic kidney but also decreased proteinuria, mesangial expansion, and expression of profibrotic factors (1,3).

The current studies are in agreement with previous preliminary reports suggesting that activation of the dopaminergic system may attenuate renal alterations in response to diabetes, although in the earlier studies, the mechanisms involved were not examined and complete assessment of nephropathy was not performed (30,38). Furthermore, these previous studies used pharmacologic agonists and antagonists, and the possibility of off-target effects cannot be excluded (38), whereas the current studies used genetic models with increased or decreased intrarenal dopamine levels. Although COMT^{-/-} null mice have global deletion of this dopamine-metabolizing gene, selective kidney transplantation confirmed a pre-eminent role for intrarenal dopamine to blunt progression of diabetic nephropathy. Unlike AADC, which is localized predominantly to the proximal tubule, COMT is expressed in multiple segments

along the nephron (39), precluding the use of Cre-lox technology to inhibit intrarenal COMT expression.

Selective depletion of intrarenal dopamine production accelerated diabetic nephropathy in our studies, consistent with alterations in intrarenal dopamine production that have been previously reported in both experimental and human diabetic nephropathy (15,36,40–42). These studies further confirm that intrarenal dopamine plays an important modulatory role in development and progression of renal injury in diabetes. Our previous studies in ptAADC^{-/-} mice indicated that these mice had increased expression and activity of the intrarenal RAS (13), which may have also been an important contributory factor to the observed increases in nephropathy.

Podocytes express dopamine receptors (43) and may be targets for dopamine. It is interesting to speculate that intrarenal dopamine may have direct effects on the glomerulus, given the close proximity of cells expressing AADC at the junction of Bowman's capsule and the proximal tubule (Fig. 8). Further in vivo and in vitro studies will be required to investigate the mechanisms of the potential protective

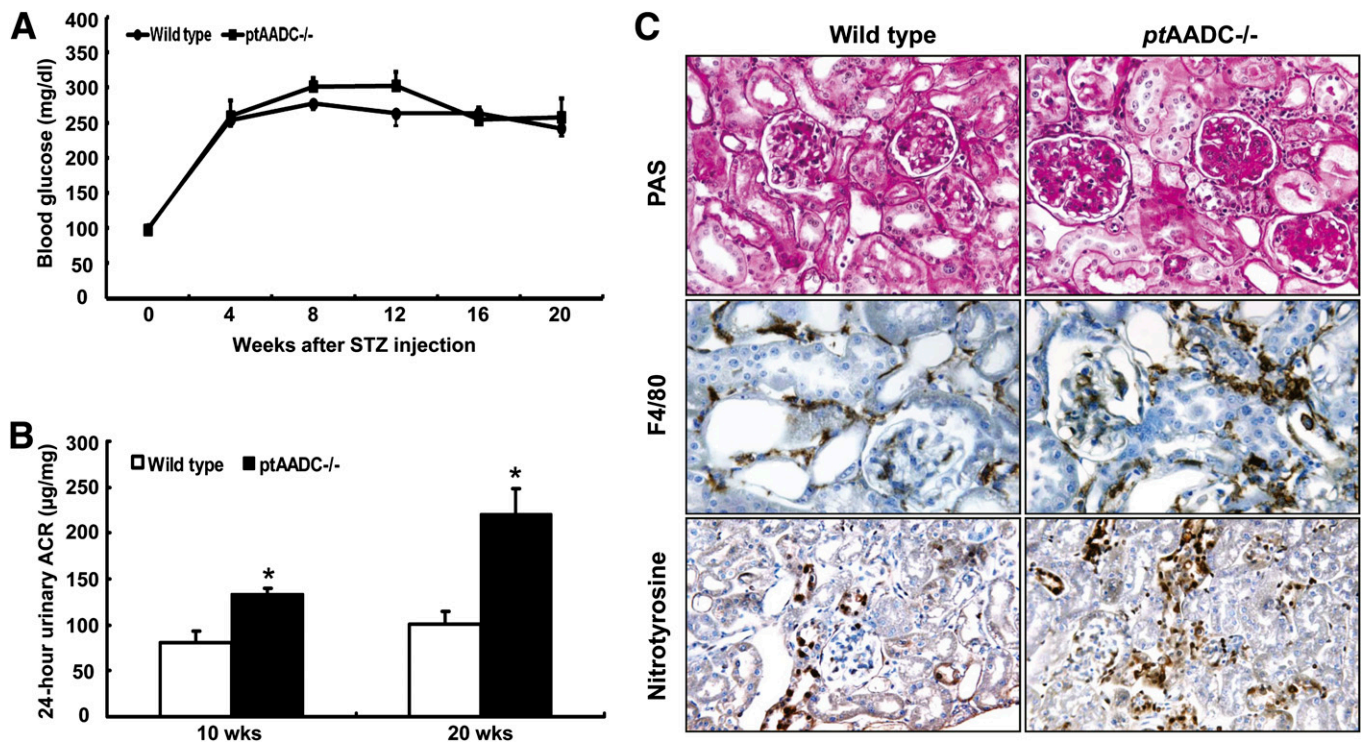


FIG. 7. STZ-induced diabetes in mice with deficient dopamine production. **A:** Hyperglycemia was equivalent in wild-type and *ptAADC*^{-/-} diabetic mice. **B:** Albuminuria was significantly increased in *ptAADC*^{-/-} diabetic mice. **P* < 0.05 compared with wild-type diabetes; *n* = 6. ACR, albumin/creatinine ratio. wks, weeks. **C:** Mesangial expansion, macrophage infiltration, and nitrotyrosine staining were increased in *ptAADC*^{-/-} diabetic mice ($\times 400$ original magnification). PAS, periodic acid Schiff. (A high-quality digital representation of this figure is available in the online issue.)

effects of dopamine and determine whether podocytes are a primary target.

In conclusion, these studies used mouse models with increased or decreased renal dopamine production to provide definitive evidence for an important role of the intrarenal dopaminergic system to modulate the development and progression of diabetic kidney injury. These results provide support that the decreased renal dopamine production reported in experimental and human diabetes may have important consequences in the underlying pathogenesis of diabetic nephropathy.

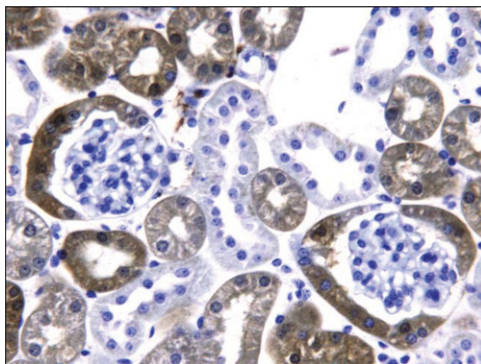


FIG. 8. Expression of AADC in wild-type mice. Note that the columnar cells of the early proximal tubule/Bowman's capsule expressing AADC are in close proximity to the glomerular tuft ($\times 400$ original magnification). (A high-quality color representation of this figure is available in the online issue.)

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No potential conflicts of interest relevant to this article were reported.

M.-Z.Z. and R.C.H. researched data and wrote the manuscript. B.Y., S.Y., H.Ya., S.W., X.F., and H.Yi. researched data. A.B.F. and G.W.M. researched data and reviewed and edited the manuscript. R.C.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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