Research Article

Altered Expression Profile of Renal α_{1D} -Adrenergic Receptor in Diabetes and Its Modulation by PPAR Agonists

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Alpha_{1D}-adrenergic receptor (α_{1D} -AR) plays important roles in regulating physiological and pathological responses mediated by catecholamines, particularly in the cardiovascular and urinary systems. The present study was designed to investigate the expression profile of α_{1D} -AR in the diabetic kidneys and its modulation by activation of peroxisome proliferator-activated receptors (PPARs). 12-week-old Zucker lean (ZL) and Zucker diabetic fatty (ZD) rats were treated with fenofibrate or rosiglitazone for 8–10 weeks. Gene microarray, real-time PCR, and confocal immunofluorescence microscopy were performed to assess mRNA and protein expression of α_{1D} -AR in rat kidney tissue. Using microarray, we found that α_{1D} -AR gene was dramatically upregulated in 22-week-old ZD rats compared to ZL controls. Quantitative PCR analysis verified a 16-fold increase in α_{1D} -AR mRNA in renal cortex from ZD animals compared to normal controls. Chronic treatment with fenofibrate or rosiglitazone reduced renal cortical α_{1D} -AR gene. Immunofluorescence staining confirmed that α_{1D} -AR protein was induced in the glomeruli and tubules of diabetic rats. Moreover, dual immunostaining for α_{1D} -AR and kidney injury molecule-1 indicated that α_{1D} -AR was expressed in dedifferentiated proximal tubules of diabetic Zucker rats. Taken together, our results show that α_{1D} -AR expression is upregulated in the diabetic kidneys. PPAR activation suppressed renal expression of α_{1D} -AR in diabetic nephropathy.

1. Introduction

Alpha₁-adrenergic receptors (α_1 -ARs) are a heterogeneous family of G-protein-coupled receptors that present in most human and animal tissues, and there are considerable variations in the expression levels of α_1 -AR subtypes in various tissues of different species [1–4]. α_1 -ARs play important roles in regulating physiological and pathological responses mediated by catecholamines, particularly in the cardiovascular and urinary systems. In addition to mediating catecholamine-induced vasoconstriction, the α_1 -ARs in vascular wall have been shown to promote proliferation and hypertrophy of arterial smooth muscle cells and adventitial fibroblasts [5, 6]. In the urinary system, the demonstration of α_1 -AR expression in human prostate, bladder muscle, and smooth muscles has led to the treatment of bladder outlet obstruction and ureteral stones, via blocking these receptors [7, 8].

Of the three α_1 -AR subtypes (α_{1A} , α_{1B} , and α_{1D}), the α_{1D} -AR has been the least studied due to difficulties in obtaining

significant expression levels and poor coupling to membrane signals due to its intracellular localization [9–11]. However, recent experiments performed in α_{1D} -AR knockout models suggest that this α_1 -AR subtype plays an important role in the overall regulation of blood pressure [12]. Additionally, Armenia et al. reported that α_{1A} - and α_{1D} -ARs are the major functional subtypes of renal α_1 -ARs in both normal and streptozotocin-induced diabetic Sprague-Dawley rats [13].

Using in situ hybridization, Kurooka et al. identified the gene expression of all three α_1 -AR subtypes in human kidney cortex [14]. They found that intense α_1 -AR mRNA staining was apparent especially in the smooth muscle of arterial walls, whereas weak staining of each of the α_1 -AR mRNAs was observed in the glomeruli and renal tubules [14]. More recently, the presence and distribution of subtypes in human renal pelvis and calyces were evaluated, and α_{1D} -AR was most dense in both followed by α_{1A} and α_{1B} [8]. However, the expression and distribution patterns of α_{1D} -AR in normal and diabetic rat kidneys remain unknown.

In the present study, we examined mRNA levels of three α_1 -AR subtypes in the kidney cortex of normal Zucker lean (ZL) and Zucker diabetic (ZD) rats by microarray and real-time PCR analyses. Confocal immunofluorescence microscopy was performed to evaluate the distribution of α_{1D} -AR in the kidney of Zucker rats. Furthermore, we investigated whether activation of peroxisome proliferator-activated receptor- α (PPAR α) or PPAR γ , known renoprotective intervention in animal models of type 2 diabetes, would affect renal expression of α_1 -AR subtypes.

2. Materials and Methods

2.1. Experimental Animals. Six-week-old male Zucker lean (ZL) and Zucker diabetic fatty (ZD) rats were purchased from Charles River Laboratories (Wilmington, MA, USA). Rats were housed in a temperature-controlled room with a 12:12-hour light-dark cycle and free access to Purina 5008 rat chow and water. Blood glucose was monitored using the Accu-chek glucometer by tail-vein blood sampling. In a previous study in which we characterized the time course of blood glucose in this model [15], we showed that blood glucose of the ZD rats began to increase at week 8, reached a peak at week 12, and remained at this higher level thereafter. The rats were housed in the animal care facility at the Morehouse School of Medicine that is AAALAC accredited. All animal protocols were approved by the Institutional Animal Care and Use Committee and were in accordance with the requirements stated in the National Institutes of Health Guide for the Care and Use Laboratory Animals.

For fenofibrate treatment, 12-week-old Zucker rats were divided into 3 experimental groups: vehicle- (0.5% carboxymethylcellulose ig) treated ZL, vehicle-treated ZD, or fenofibrate- (150 mg/kg/day ig) treated ZD (F-ZD) rats for 10 weeks. For rosiglitazone treatment, 12-week-old ZD animals were treated with rosiglitazone (10 mg/kg/day in drinking water) or vehicles for 8 weeks.

2.2. Isolation of Glomeruli. Glomeruli were isolated from the kidney cortex of ZL and ZD rats at the age of 7, 12, and 20–22 weeks, respectively, by a modified procedure as described previously [16]. Briefly, the rats were anesthetized and the kidneys were rapidly removed and placed in Hanks' balanced salt solution (HBSS) at pH 7.4. The renal cortex was dissected and cut into small pieces with a surgical blade. Glomeruli were isolated by passing the tissues successively through calibrated sieves (pore size: 200, 125, and 65 μ m) and rinsed with HBSS. Isolated glomeruli, collected on the 65 μ m sieve, were resuspended in HBSS. They were devoid of Bowman's capsule. Tubular fragments were less than 3% of the total number of isolated glomeruli [16].

2.3. RNA Extraction. Total RNA was prepared from isolated glomeruli or kidney cortex by using ultrapur TRIZOL reagent according to the manufacturer's instructions (GIBCO-BRL, Grand Island, NY). The quality of the RNA samples was assessed using the Agilent 2100 Bioanalyzer (G2938A). RNA concentrations were determined spectrophotometrically (absorbance at 260 and 280 nm).

2.4. Microarray Analysis. Seven hundred and fifty nanograms of total RNA per sample were used for cRNA synthesis and amplification. Agilent Spike-In control RNA was included as an internal control. Cyanine-3-(Cy3-) labeled cRNA was purified and hybridized to Agilent Whole Rat Genome 44k Oligo Microarray chips (p/n G2519F-14870, Agilent Technologies) according to the manufacturer's instructions. The processed microarrays were scanned with the Agilent G2565BA DNA Microarray Scanner (p/n G2505-A). The scanned images were analyzed with Agilent Feature software (version 9.5.1.1) using default parameters. The resulting text files were loaded into the Agilent GeneSpring GX software (version 7.3) for further analysis. Gene expression values of all datasets were normalized using median normalization. Significantly differentially expressed α -adrenergic receptor genes among ZL, ZD, and F-ZD groups were identified by a threshold of ≥ 2 fold change and $P \leq 0.05$.

2.5. Real-Time PCR for α_{1A} -, α_{1B} -, and α_{1D} -AR mRNA Expression. Reverse transcription was performed on equal amounts of total RNA by using random hexanucleotide primers to produce a cDNA library for each sample. Real-time PCR reactions were run on an iCycler iQ Real-Time PCR Detection System by using Taqman Universal PCR Master Mix (Applied Biosystems, P/N 4304437). α_{1A} -, α_{1B} -, and α_{1D} -AR and β -actin gene-specific Taqman probe and primer sets were obtained from Applied Biosystems as Assays-on-Demand (AOD) gene expression products. The AOD identification numbers were Rn00567876 for α_{1A} -AR, Rn01471343 for α_{1B} -AR, Rn00577931 for α_{1D} -AR, and 4331182 for rat β -actin endogenous control. Each sample was run in triplicate, and the comparative threshold cycle (C_t) method was used to quantify fold increase ($2^{-\Delta\Delta C_t}$) compared with controls.

2.6. Immunostaining. For immunofluorescent staining, 5 μ m thick cryostat sections of OCT-embedded kidney samples were used. To study the localization of α_{1D} -AR in the rat kidney, the sections were incubated with a mixture of two antibodies overnight: rabbit anti- α_{1D} -AR antibody (1:100, Sigma-Aldrich, St. Louis, MO), mouse anti- α -smooth muscle actin (α -SMA, 1:100, Santa Cruz Biotechnology, Dallas, TX), or goat anti-kidney injury molecule-1 (Kim-1, 1:100, R&D Systems, Minneapolis, MN). As a negative control, the sections were exposed to nonimmune IgG (in replacement of primary antibodies) with the same secondary antibodies, and no specific staining occurred. The sections were observed and imaged by Leica confocal microscope.

2.7. Statistical Analysis. Data are expressed as mean $\pm$ SEM. Student's *t*-test was used for comparison between the two groups. Comparisons among multiple groups were performed by one-way ANOVA and Newman-Keuls post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Differential Gene Expression of Alpha-Adrenergic Receptor Subtypes in the Diabetic Kidney. We performed microarray

TABLE 1: Alpha-adrenergic receptor gene expression in the kidney cortex of Zucker lean (ZL), untreated Zucker diabetic fatty (ZD), and fenofibrate-treated diabetic fatty (F-ZD) rats.

Accession number	Gene name	mRNA expression ZL	ZD versus ZL		F-ZD versus ZD	
			Fold change	P value	Fold change	P value
NM_017191	α_{1A} -adrenergic receptor (α_{1A} -AR)	10.4 $\pm$ 2.9	NA	NA	NA	NA
NM_016991	α_{1B} -adrenergic receptor (α_{1B} -AR)	1372.2 $\pm$ 101.8	1.49	NS	1.26	NS
NM_024483	α_{1D} -adrenergic receptor (α_{1D} -AR)	273.3 $\pm$ 142.9	6.53	<0.001	-2.75	<0.001
NM_012739	α_{2A} -adrenergic receptor (α_{2A} -AR)	53.2 $\pm$ 15.2	-1.09	NS	1.02	NS
NM_138505	α_{2B} -adrenergic receptor (α_{2B} -AR)	116.8 $\pm$ 22.8	-1.18	NS	1.05	NS
NM_138506	α_{2C} -adrenergic receptor (α_{2C} -AR)	33.1 $\pm$ 7.9	1.19	NS	1.07	NS

Data are shown in two ways: mRNA expression level presenting the relative abundance of different alpha-adrenergic receptor subtypes in normal rat kidney cortex and fold change showing the differential expression of these genes among ZL, ZD, and F-ZD groups. There is significant ($P < 0.001$) upregulation in renal expression of α_{1D} -AR gene in the ZD animals, which is markedly prevented by fenofibrate treatment ($P < 0.001$). NA: not analyzed due to its low expression level in ZL, ZD, and F-ZD animals; NS: not significant.

analysis to assess gene expression levels of α -adrenergic receptor subtypes in the kidney cortex of 22-week-old normal ZL (blood glucose: 108 $\pm$ 8 mg/dL) and diabetic ZD (blood glucose: 425 $\pm$ 35 mg/dL) rats. The expression profile of each experimental group was determined in three animals per group. Table 1 shows gene expression levels of α_1 - and α_2 -adrenergic receptor subtypes detected in rat kidney tissue. The rank order of expression levels of the three α_1 -AR mRNAs in normal rat kidney cortex was $\alpha_{1B} > \alpha_{1D} > \alpha_{1A}$. Among these genes, α_{1D} -AR was significantly upregulated in the kidney cortex of diabetic animals. Compared to normal ZL controls, renal cortical α_{1D} -AR gene was increased by 553% in 22-week-old ZD rats (Table 1).

To verify the relative transcript levels of α_{1A} -, α_{1B} -, and α_{1D} -AR subtypes derived from the microarray experiment, we performed quantitative real-time PCR (qPCR) assay. The relative expression levels of α_{1A} -, α_{1B} -, and α_{1D} -AR mRNAs normalized against β -actin in rat kidney cortex are shown in Figure 1. In consistency with the microarray results, qPCR analysis showed that renal cortical α_{1D} mRNA was increased by 16-fold in 22-week-old ZD rats compared to ZL controls.

3.2. Activation of PPAR α or PPAR γ Inhibited the Upregulation of α_{1D} -AR mRNA Expression in the Diabetic Kidneys. We have previously shown that PPAR activation protects against kidney injury in Zucker diabetic fatty rats [15, 17]. Here, we further examined the effect of PPAR activation on the expression level of α_1 -AR mRNAs in the diabetic kidneys. Gene microarray analysis revealed that PPAR α activation inhibits the upregulation of α_{1D} gene in the diabetic kidneys. Chronic administration of fenofibrate, a PPAR α agonist, resulted in a decrease in α_{1D} -AR mRNA by 64% compared to vehicle-treated ZD animals (Table 1). qPCR analysis confirmed a reduction of α_{1D} -AR mRNA in the kidney of F-ZD rats (Figure 1). In contrast, both microarray and qPCR analyses indicated that mRNA expression of α_{1A} - and α_{1B} -AR subtypes was not affected by fenofibrate in the diabetic kidneys.

Additionally, the effect of PPAR γ activation on gene expression of α_1 -AR subtypes was examined in the ZD rats after rosiglitazone treatment. Compared to vehicle-treated ZD rats, renal cortical α_{1D} -AR mRNA was significantly lower when rosiglitazone was administered for 8 weeks (Figure 2).

Similar to PPAR α activation, α_{1A} - and α_{1B} -AR mRNA expression in the diabetic rats was not affected by rosiglitazone treatment.

3.3. Glomerular and Cortical Expression of α_{1D} -AR mRNA in Zucker Rats of Various Ages. In a previous study [15], we showed that blood glucose levels were not different between normal ZL and diabetic ZD rats at the age of 7 weeks. Blood glucose of the ZD rats began to increase at week 8, reached a peak at week 12, and remained at this higher level thereafter. To analyze the temporal pattern of renal expression of α_{1D} -AR receptors, renal cortical and glomerular α_{1D} -AR mRNA levels were compared in the Zucker rats at the ages of 7, 12, and 20–22 weeks. As shown in Figure 3, ZD rats at week 7 had slightly lower α_{1D} -AR mRNA level in the glomeruli compared to their ZL littermates, whereas renal cortical α_{1D} -AR mRNAs were not different between the two groups. At the age of 12, both renal glomerular (2.4-fold) and cortical (1.7-fold) α_{1D} -AR mRNA levels were significantly higher in the ZD animals, which were further increased by 3.4-fold and 12.9-fold, respectively, at the age of 20–22 weeks.

3.4. Expression and Distribution of α_{1D} -AR Protein in the Kidney of Zucker Rats. We performed immunofluorescence staining to correlate α_{1D} -AR gene expression results with its protein level and distribution in the kidney of Zucker animals. As expected, α_{1D} -AR protein was clearly detected in the renal arteries and arterioles in both normal and diabetic animals (Figure 4). The intense staining was primarily in the smooth muscle of renal arterial walls as evidenced by its colocalization with α -SMA. In normal ZL rats, weak α_{1D} -AR staining was also detected in the glomeruli, whereas there was no obvious staining in the renal tubules (Figure 4). In consistency with the mRNA expression results, α_{1D} -AR protein was largely induced in the diabetic ZD kidneys. Immunofluorescence staining identified increased α_{1D} -AR signal in the glomeruli of diabetic rats, which was partially colocalized with α -SMA (Figure 4). Moreover, intense tubulointerstitial staining of α_{1D} -AR was apparent in the diabetic kidneys. As shown in Figure 5, α_{1D} -AR was expressed in both tubular epithelial cells and activated interstitial fibroblasts, which was positive for α -SMA staining.

FIGURE 1: mRNA expression of renal cortical α_{1A} -, α_{1B} -, and α_{1D} -AR subtypes was measured by quantitative real-time PCR in 22-week-old Zucker lean (ZL), vehicle-treated Zucker diabetic (ZD), and fenofibrate-treated Zucker diabetic (F-ZD) rats. Fold changes of α_{1A} - (a), α_{1B} - (b), and α_{1D} - (c) AR genes were calculated using β -actin as an internal control. Values are mean $\pm$ SEM. $n = 5$ -6 animals/group. *** $P < 0.001$ versus ZL; ### $P < 0.001$ versus ZD group.

To further characterize α_{1D} -AR expression in renal tubules of diabetic animals, we performed double staining for α_{1D} -AR and Kim-1, a sensitive tubular injury marker. Dual labeling revealed a spatial relationship between Kim-1 and α_{1D} -AR in the diabetic kidneys. As shown in Figure 6, virtually all dilated tubules expressing α_{1D} -AR were also Kim-1-positive, suggesting that α_{1D} -AR was expressed in the injured dedifferentiated proximal tubules. In these tubules, α_{1D} -AR expression was predominantly cytoplasmic, whereas Kim-1 staining was prominent at the apical membrane.

4. Discussion

In this study, the expression and distribution of α_{1D} -AR mRNA and protein were determined by the gene microarray, qPCR, and confocal immunofluorescence analyses. Although mRNA expression of all three α_1 -AR subtypes ($\alpha_{1B} > \alpha_{1D} > \alpha_{1A}$) was detected in rat kidney cortex, only α_{1D} -AR gene was massively upregulated in the diabetic animals. Moreover, diabetes-related increase in α_{1D} -AR mRNA was inhibited

when the ZD rats were treated with fenofibrate or rosiglitazone. Immunostaining for α_{1D} -AR confirmed that intense α_{1D} -AR staining was apparent especially in the smooth muscle of arterial walls in both normal and diabetic kidneys. Weak α_{1D} -AR protein staining was detected in the glomeruli of normal ZL controls, but there was no obvious staining in the normal tubular epithelium. In consistency with the gene expression results, α_{1D} -AR protein was significantly increased in the glomeruli and proximal tubules of diabetic animals.

The expression of α_1 -AR subtype mRNAs has previously been studied in various animal and human organs, and the predominant subtype mRNA expressed differs among species and organs. For example, Kurooka et al. reported that α_{1A} -AR gene was detected more than α_{1B} -AR or α_{1D} -AR in human kidney cortex [14]. In contrast, Karabacak et al. [8] recently evaluated α_1 -AR subtype protein expression in human renal pelvis and calyx tissues and found that α_{1D} -AR was most dense in both followed by α_{1A} - and α_{1B} -AR subtypes, respectively, where the rate of α_{1B} -AR was significantly lower than the other two. In the rat kidney, it was reported that the α_{1B} -AR is predominant when detected by

FIGURE 2: Effect of PPAR γ activation on renal expression of α_{1A} -, α_{1B} -, and α_{1D} -AR subtype mRNAs. Bar graphs present the real-time PCR results of renal cortical α_{1A} - (a), α_{1B} - (b), and α_{1D} - (c) AR mRNAs in 20-week-old ZD rats following treatment with rosiglitazone for 8 weeks. Values are mean $\pm$ SEM. $n = 5-6$ animals/group. # $P < 0.05$ versus vehicle-treated ZD group.

FIGURE 3: mRNA expression of α_{1D} -AR in the glomeruli and cortex of normal ZL and diabetic ZD rats at different time points. α_{1D} -AR gene was progressively increased in both glomeruli (a) and cortex (b) of ZD rats compared to normal ZL littermates. mRNA fold changes of α_{1D} -AR were calculated using β -actin as an internal control. Values are mean $\pm$ SEM. $n = 5-6$ animals/group. * $P < 0.05$, ** $P < 0.01$ versus ZL control group.

FIGURE 4: Representative confocal immunofluorescence images of α_{1D} -AR and α -smooth muscle actin (α -SMA) in the glomeruli of ZL and ZD rats. In normal control, colocalization of α_{1D} -AR (green) with α -SMA (dark blue) was apparent in the renal vasculature (white arrow), whereas weak staining of α_{1D} -AR was detected in the glomeruli. An increase in α_{1D} -AR staining in the diabetic glomeruli was accompanied by an increase in α -SMA signal.

FIGURE 5: Increased tubulointerstitial α_{1D} -AR staining in the kidney of 20-week-old Zucker diabetic rats. In the diabetic kidneys, an induction of α_{1D} -AR protein (green) was detected in the epithelium of dilated tubules as well as the surrounding interstitial cells, which are α -SMA-positive (dark blue). Images in (a'), (b'), and (c') are enlarged from the boxed areas in (a), (b), and (c).

FIGURE 6: Representative confocal images of double staining for α_{1D} -AR and Kim-1 in the tubules of 20-week-old Zucker diabetic rats. Colocalization of α_{1D} -AR (green) and Kim-1 (red) was seen in the dilated proximal tubules of diabetic rats. Images in (a'), (b'), and (c') are enlarged from the boxed areas in (a), (b), and (c).

a radioligand binding assay [18] and RNase protection assay [19]. In consistency with these findings, we confirmed the expression of all three α_1 -AR subtype mRNAs ($\alpha_{1B} > \alpha_{1D} > \alpha_{1A}$) in rat kidney cortex by microarray and qPCR analyses.

Of the three α_1 -AR subtypes, the α_{1D} -AR has been the least studied due to difficulties in obtaining significant expression levels and poor coupling to membrane signals due to its intracellular localization [9–11]. However, recent experiments performed in α_{1D} -AR knockout models suggest that this α_1 -AR subtype plays an important role in the overall regulation of blood pressure [12]. Moreover, Armenia et al. reported that α_{1A} - and α_{1D} -ARs are the major functional subtypes of renal α_1 -ARs in both normal and streptozotocin-induced Sprague-Dawley rats [13]. Interestingly, among the three α_1 -AR subtypes detected in rat kidney cortex, we found that α_{1D} -AR mRNA was markedly increased by 16-fold in the diabetic kidneys. Additionally, diabetes-associated upregulation of α_{1D} -AR mRNA expression was inhibited when the diabetic animals were treated with PPAR agonists, known renoprotective interventions. Therefore, previous evidences and present results strongly suggest that α_{1D} -AR may play an important role in renal physiology and/or pathophysiology.

Diabetic nephropathy is characterized by a series of ultrastructural changes, including glomerular and tubular hypertrophy, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis. We have previously demonstrated a progressive loss of renal function in the diabetic animals as evidenced by an increase in urinary protein to creatinine ratio in the ZD rats between the ages of 7 and 20 weeks [15]. In the current study, we further observed a gradual increase in cortical and glomerular α_{1D} -AR mRNA expression during disease progression in the diabetic animals. We speculate that increased α_{1D} -AR may play a role in the development of diabetic renal hypertrophy and fibrosis. In fact, a role for α_{1D} -AR in vascular hypertrophy and remodeling has been suggested by a recent report that a decrease in the lumen area and an increase in the wall thickness of arteries in hypoxic pulmonary hypertension were strongly inhibited in α_{1D} -AR knockout mice [5]. In this study, we evaluated the expression and distribution of α_{1D} -AR protein in the kidneys of ZL and ZD rats by immunofluorescence staining. As expected, intense α_{1D} -AR staining was apparent especially in the smooth muscle of arterial walls in both normal and diabetic kidneys. Compared to low expression level of α_{1D} -AR protein in the glomeruli of normal ZL controls, ZD rats demonstrate a significant increase in α_{1D} -AR signal. Moreover, ZD kidneys displayed strong tubulointerstitial α_{1D} -AR signal within fibrotic areas. α_{1D} -AR protein was expressed in both tubular epithelial cells and interstitial cells. A colocalization of α_{1D} -AR with α -SMA indicates that α_{1D} -AR expressing interstitial cells are myofibroblasts. Future work in the field could be necessary to establish the exact role of α_{1D} -AR in activation and proliferation of renal interstitial fibroblasts.

Another interesting novelfinding in our set of data reported here is the α_{1D} -AR expression in tubular epithelial cells of diabetic kidneys. Although there was no obvious staining in the normal tubules, α_{1D} -AR staining was apparent in the dilated tubules in the fibrotic areas. To further

characterize α_{1D} -AR expression in renal tubules of diabetic animals, the spatial relationship between tubular α_{1D} -AR and Kim-1, a tubular injury marker, was studied by double staining. In tubulointerstitial fibrotic areas, virtually all dilated tubules expressing α_{1D} -AR were also Kim-1-positive. The selective increased expression by dedifferentiated epithelial cells and activated interstitial fibroblasts supports the potential importance of α_{1D} -AR in renal tubulointerstitial injury. Moreover, recent studies on the pulmonary circulation suggest that catecholamines may participate in the excessive muscularization and fibrosis of the arteries through ERK1/2 signaling pathway in hypoxic pulmonary hypertension [5, 20–22]. Therefore, further studies are warranted to evaluate the functional consequences of α_{1D} -AR induction in diabetic kidney injury.

In summary, the current study highlights mRNA expression of the three α_1 -AR subtypes in rat kidney cortex. An increase in renal expression of α_{1D} -AR mRNA and protein was associated with glomerular and tubulointerstitial injury in diabetic nephropathy. Chronic treatment with PPAR agonists prevents the increase in α_{1D} -AR mRNA in the diabetic kidneys. These findings may provide new insights into the prevention and early management of diabetic kidney disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding this paper.

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