Life Sciences 92 (2013) 1174–1179

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Renal GLUT1 reduction depends on angiotensin-converting enzyme inhibition in diabetic hypertensive rats

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ARTICLE INFO

Article history:
Received 2 February 2013
Accepted 1 May 2013

Keywords:
Angiotensin-converting enzyme inhibitors
Diabetes mellitus
Diabetic nephropathy
Glucose transporter proteins
Hypertension
Streptozotocin

ABSTRACT

Aims: Angiotensin-converting enzyme (ACE) inhibitors are used in diabetic kidney disease to reduce systemic/intra-glomerular pressure. The objective of this study was to investigate whether reducing blood pressure (BP) could modulate renal glucose transporter expression, and urinary markers of diabetic nephropathy in diabetic hypertensive rats treated with ramipril or amlodipine.
Main methods: Diabetes was induced in spontaneously-hypertensive rats (~210 g) by streptozotocin (50 mg/kg). Thirty days later, animals received ramipril 15 μg/kg/day (R, n = 10), or amlodipine 10 mg/kg/day (A, n = 8,) or water (C, n = 10) by gavage. After 30-day treatment, body weight, glycaemia, urinary albumin and TGF-β1 (enzyme-linked immunosorbent assay) and BP (tail-cuff pressure method) were evaluated. Kidneys were removed for evaluation of renal cortex glucose transporters (Western blotting) and renal tissue ACE activity (fluorometric assay).
Key findings: After treatments, body weight ($p = 0.77$) and glycaemia ($p = 0.22$) were similar among the groups. Systolic BP was similarly reduced ($p < 0.001$) in A and R *vs.* C (172.4 ± 3.2; 186.7 ± 3.7 and 202.2 ± 4.3 mm Hg; respectively). ACE activity (C: 0.903 ± 0.086; A: 0.654 ± 0.025, and R: 0.389 ± 0.057 mU/mg), albuminuria (C: 264.8 ± 15.4; A: 140.8 ± 13.5 and R: 102.8 ± 6.7 mg/24 h), and renal cortex GLUT1 content (C: 46.81 ± 4.54; A: 40.30 ± 5.39 and R: 26.89 ± 0.79 AU) decreased only in R ($p < 0.001$, $p < 0.05$ and $p < 0.001$; respectively).
Significance: We concluded that the blockade of the renin–angiotensin system with ramipril reduced early markers of diabetic nephropathy, a phenomenon that cannot be specifically related to decreased BP levels.

Introduction

Prevention and treatment of diabetic nephropathy aim at reducing blood pressure levels using angiotensin-converting enzyme (ACE) inhibitors and attaining low HbA1c levels (The Diabetes Control and Playstation Trial Research Group, 1993; Lewis et al., 1993). These approaches are based on clinical trials and experimental research showing that reduction of systemic blood pressure is important. Notably, ACE inhibitors additionally act to reduce intraglomerular pressure, in ways not shared by other anti-hypertensive drugs (Christensen et al., 2001; Singh et al., 2003; Souza et al., 2008; Zatz et al., 1986).

The process of renal glucose reabsorption takes place in the epithelial cells of the proximal tubule, involving Na^+-glucose transporters (SGLTs) and facilitative diffusion transporters (GLUTs) (Thorens, 1996). The bulk of filtered glucose is reabsorbed in the early S1 segment, where the low-affinity/high-capacity glucose transporters, SGLT2 and GLUT2 coexpress in the luminal brush border membrane and in the basolateral membrane; respectively. Diabetes is characterized by cortical GLUT2 gene expression increases (Dominguez et al., 1992, 1994; Marks et al., 2003; Schaan et al., 2005b), which are important for renal glucose reabsorption maintenance under such condition, as high blood and interstitial glucose concentrations may reduce the outwardly directed glucose gradient from tubule to blood (Dominguez et al., 1994). GLUT1 protein is also present in the outer renal cortex, where it is related to mesangial cells (Li et al., 2001). Previously we showed that increased expression of cortical GLUT1 (mesangial cells) and GLUT2 (S1 tubular cells) is involved in the development of diabetic nephropathy (Schaan et al., 2001; Vestri et al., 2001). Urinary albumin and transforming growth factor-β1 (TGF-β1) are increased simultaneously with high GLUT1 and GLUT2 protein in the renal cortex of streptozotocin (STZ)-treated diabetic rats (Schaan et al., 2001), and all these changes are magnified by the association with arterial hypertension (Schaan et al., 2005a). The well-known diabetes-induced GLUT2 overexpression and the further

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http://dx.doi.org/10.1016/j.lfs.2013.05.001

rise that hypertension can promote may cause (Freitas et al., 2005, 2007; Schaan et al., 2005b), in addition to hyperglycaemia (Schaan et al., 2005a), a further elevation in the interstitial renal glucose concentration, and more glucose is taken up by mesangial cells through GLUT1.

Since high glucose concentration can increase mesangial angiotensin II generation and oxidative stress (Cristovam et al., 2008; Takao et al., 2011; Vidotti et al., 2004), and angiotensin II subsequently can determine GLUT1 overexpression in mesangial cells (Nose et al., 2003), we investigated the inhibition of the renin–angiotensin system on cortical renal GLUT1 and GLUT2 expression in vivo, for the first time showing that ramipril could down-regulate these glucose transporters in the kidney of STZ-treated diabetic hypertensive rats (Souza et al., 2008). However, urinary albumin and TGF-β1 levels were not reduced by this strategy, and we could not discriminate whether the effects upon glucose transporters were caused by reducing blood pressure levels or by blocking the renin–angiotensin system, as we did not compare ramipril with non-renin angiotensin system inhibitors.

We hypothesized that ACE-inhibition could be beneficial in treating diabetic nephropathy beyond its expected role in blood pressure reduction, as it could modulate renal glucose transporters, which are involved in the pathogenesis of this condition. The objective of this study was to investigate whether reducing blood pressure levels with ramipril and amlodipine in diabetic hypertensive rats could modulate renal glucose transporter expression, as well as urinary markers of diabetic nephropathy.

Materials and methods

Samples and experimental conditions

All animal experimentation procedures followed the Guide for the Care and Use of Laboratory Animals and Colégio Brasileiro de Experimentação Animal. (Colégio Brasileiro de Experimentação Animal, 1991; NIH, 1996) The study was approved by the Research Ethics Committee of Instituto de Cardiologia do Rio Grande do Sul, Brazil.

Experiments were performed in 2-month-old male spontaneously-hypertensive rats (SHR, Animal House of the Coordenação de Produção e Experimentação Animal, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS, Brazil), weighing ~210 g, acclimatized for 1 week. Animals were allowed free access to water and standard rodent chow diet (Nuvilab CR-1, Nuvital, Curitiba, PR, Brazil), 12-hour light/12-hour dark cycle, 6 a.m./6 p.m. and stable temperature (23 ± 2 °C) conditions.

Animals were fasted overnight and rendered diabetic (D) by a single injection of streptozotocin, 50 mg/kg (Sigma Chemical Co., USA) into the tail vein. STZ was dissolved in citrate buffer, pH 4.5, and injected slowly, 5 min after its dilution. Diabetes was defined as non-fasting glycaemia >250 mg/dL (test strips, Advantage, Roche, USA) in tail vein blood 48 h after streptozotocin injection. Animals were maintained for 30 days in individual cages with free access to tap water and standard rat chow. After that period, they were treated, for 30 days, with water (C; n = 10), amlodipine (15 mg · kg^{-1} · day^{-1}, A; $n = 8$) or ramipril (1 mg · kg^{-1} · day^{-1}, R; n = 10), administered by gavage at the same volume. Afterwards, 24-h urine was collected in metabolic cages for glucose, albumin and TGF-β1 analyses.

Blood pressure (BP) was measured using a tail-cuff system (Model 229, Amplifier with automatic cuff inflation, IITC Life Science Inc.) at the end of treatments. Animals were placed in a restrainer for 15 min, a cuff was attached to their tail and BP was then recorded. Animals were subjected to an adaptation period of one week in order to get used to that measurement procedure, which was repeated on three non-consecutive days in the same week. Blood pressure measures obtained from day 4 were considered valid.

Rats were then anaesthetized with sodium pentobarbitone (25 mg/kg body weight, *iv*), their kidneys were perfused with Hanks' buffer to eliminate the intravascular blood content, and removed (60 days of diabetes, 30 days after ramipril, amlodipine or placebo treatment). Renal outer cortex was dissected and the tissue fragments were weighed and frozen at −70 °C for further analysis (GLUT1 and GLUT2 protein content).

Glucose, albumin and TGF-β1

Glycaemia (test strips) was evaluated 48 h and 60 days after STZ injection. Urinary glucose was measured using the colorimetric enzymatic test (commercial kit, Merck, Germany).

Samples for the measurement of urinary albumin were collected without preservatives and stored at −70 °C after centrifugation. Albuminuria was measured by a quantitative direct enzyme-linked immunosorbent assay (ELISA, Nephrat, Exocell Inc., USA), using an anti-rat albumin antibody. The quantification range for albuminuria was 0.156–10 mg/dL. Samples were diluted 1:2. Results are reported as mg/24 h.

Urinary TGF-β1 was assayed by solid phase ELISA (R&D Systems, UK). Urine samples were collected on ice and centrifuged at 10 000 rpm for 30 min at 4 °C. Supernatant was removed and stored at −70 °C. On the day of the assay, samples (0.5 mL) were acidified to pH of 2–3 with 100 μL 1 N HCL for 10 min and then re-neutralized to pH 7–8 with 100 μL of 1.2 N NaOH/0.5 M HEPES. Results are reported as pg/24 h. The mean intra- and interassay coefficients of variation were 2.0 and 13.1%; respectively.

Angiotensin-converting enzyme activity in renal tissue

ACE activity was determined by fluorometric assay (Santos et al., 1985). One kidney was quickly harvested, rinsed, blotted and homogenized in 0.4 M sodium borate buffer, pH 7.2. Supernatants from homogenized tissues (20 μL) were incubated with 490 or 480 μL of assay buffer containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer and 0.9 M NaCl, pH 8.3, for 15 or 30 min at 37 °C. The reaction was stopped by the addition of 1.2 mL of 0.34 M NaOH. The product, peptide His-Leu release, was measured fluorometrically at 365-nm excitation and 495-nm emission, using a fluorescence spectrometer (Shimadzu, RF 1501, Japan). *o*-phthaldialdehyde (100 μL, 20 mg/mL) in methanol was added, and after 10 min the solution was acidified with 200 μL 3 N HCl and centrifuged at 3000 rpm for 10 min at room temperature. In order to correct for the intrinsic fluorescence of the tissues, time zero blanks were prepared by adding tissue after NaOH. Assay sensitivity was ≤0.02 nmol · mg $tissue^{-1}$ · min^{-1}; fluorescence intensity was linear with the concentration of His-Leu released from 0.02 to 15 nmol · mg $tissue^{-1}$ · min^{-1}. The results are reported as nmol His-Leu · min^{-1} · mg $protein^{-1}$, measured with Bradford's method (bovine serum albumin as standard) (Bradford, 1976).

GLUT1 and GLUT2

Renal cortex was analyzed for GLUT1 and GLUT2 protein content. Tissue samples were mechanically processed and cell membranes were disrupted by sonication (Unique, Indaiatuba, SP, Brazil) in 10 w/v buffer (10 mM Tris–HCl, 1 mM EDTA, and 250 mM sucrose, pH 7.4, containing 5 mg/mL aprotinin), and centrifuged at 3000 ×*g* for 15 min. The supernatant was centrifuged at 12 000 ×*g* for 20 min, and the pellet was suspended as a plasma membrane enriched fraction (PM). Western blot analysis was then performed as previously described (Freitas et al., 2005; Schaan et al., 2005a). Briefly, equal amounts of membrane protein (150 μg) were subjected to SDS-PAGE (10%) and transferred to nitrocellulose membrane. After blocking with non-fat-milk, membranes were incubated with the specific antibody (#07-1401 and #07-1402, Millipore, Billerica, USA), 1:3000, followed by washing with saline phosphate buffer (PBS)/0.05% Tween 20 and incubation with an HRP-conjugated secondary antibody (AP307P, Millipore, Billerica, USA), 1:10 000.

The membrane was rewashed and incubated in the dark with peroxidase substrate (ECL kit, GE Healthcare, New York, USA) for 1–3 min and exposed to ultrasensitive radiographic film (Kodak, Frankfurt, Germany) for 1 h. Blot intensity was quantified by optic densitometry using Scion Image software. After that, membranes were reprobed with anti-β-actin antibody (Monoclonal anti-β-actin antibody AC-74, A2228, Sigma-Aldrich), and GLUT1 and GLUT2 values were normalized by the respective β-actin value. Results were expressed as arbitrary units (AU/μg protein).

Histology

In the eleven animals as described above, 3 untreated (C), 4 ramipril-treated (R) and 4 amlodipine-treated (A) rats, kidney histological analysis was performed searching for fibrosis. After the treatments, rats were anaesthetized and killed by exsanguination, the right kidney was removed and fixed in 10% formalin, dehydrated and included in paraffin. Five μm tissue sections were stained with sirius red. The *Image-Pro Plus 6.0.0.206* software was used to capture images and quantify the corticomedullary junction collagen areas stained in red. This was performed using a light microscope (BX41, Olympus, Tokyo, Japan) in original magnification. Results are presented as the ratio of fibrotic area to the rest of the kidney photomicrograph.

Data analysis

Data are reported as means ± SEM or median and 25–75 percentiles. Statistical significance was calculated by one-way ANOVA, *post hoc* Tukey. Urinary albumin and TGF-β1 data were log-transformed before analysis. $p < 0.05$ was considered significant, with the critical value of p being one-sided. The Statistical Package for Social Sciences (version 15.0, SPSS, Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the baseline characteristics of the animals studied and the metabolic effects of streptozotocin, amlodipine and ramipril during the experiment. During the entire experimental period, body weights were similar among groups. Neither group of rats gained weight, as expected, because of their non-treated diabetic condition. Moreover, increased glycaemia, urinary glucose excretion and diuresis were determined by streptozotocin injection, with no significant difference in these variables among groups, except for glycaemia in group A at the end of the experiment, as compared to group R.

The results for systolic BP and renal tissue ACE activity are reported in Fig. 1, panels A and B; respectively. Systolic BP was high in all groups studied, as expected for hypertensive rats, and decreased by ~10% ($p < 0.007$) in animals treated with both anti-hypertensive drugs amlodipine (A) or ramipril (R). No significant difference in BP was observed between groups A and R. Both amlodipine and ramipril treatment decreased renal tissue ACE activity (17% and 63%; respectively), as compared to C. Furthermore, compared to group A, the ACE activity in group R was reduced ($p = 0.022$).

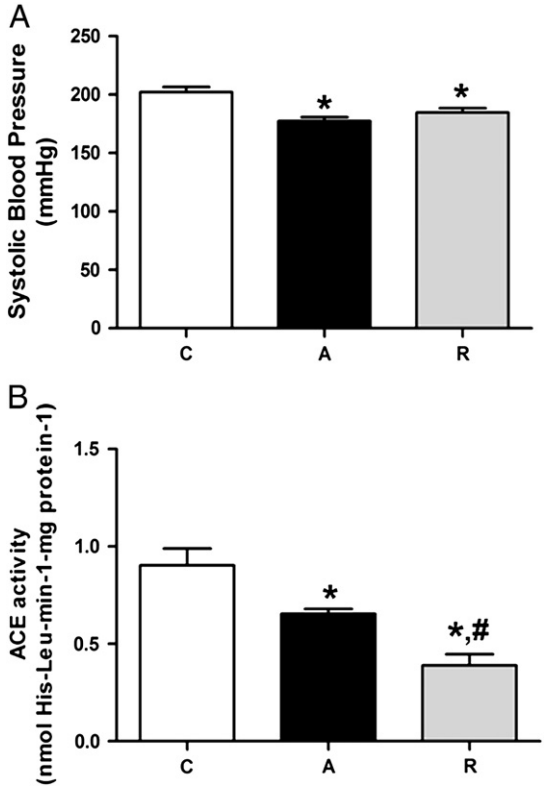

Fig. 1. Systolic blood pressure (SBP) (panel A) and renal angiotensin-converting enzyme (ACE) activity (panel B) in the groups studied. *$p < 0.05$ *vs.* C; #$p < 0.05$ *vs.* A. Data are reported as means ± SEM. One way ANOVA; *post hoc* Tukey.

Both urinary albumin (Fig. 2, panel A) and TGF-β1 (Fig. 2, panel B) were high in group C 60 days after the STZ injection. Albuminuria decreased in rats treated with both anti-hypertensive drugs tested (A and R *vs.* C, $p < 0.001$), with the lowest levels being observed in R (R *vs.* A rats, $p = 0.025$). Urinary TGF-β1 was increased in group A as compared to C ($p = 0.003$). Importantly, TGF-β1 decreased in rats of group R as compared to C rats ($p = 0.028$).

Fig. 3 shows that only ramipril caused a significant reduction in renal cortex GLUT1 content (C: 46.81 ± 4.54; A: 40.30 ± 5.39 and R: 26.89 ± 0.79 AU, $p = 0.001$, panel A). Cortical renal GLUT2 content was also lower only in R rats (C: 0.68 ± 0.04, A: 0.79 ± 0.06 and R: 0.48 ± 0.03 AU, $p = 0.015$, panel B).

Fig. 4 depicts representative photomicrographs of picro-sirius red stained corticomedullary sections (panel A, group C; panel B, group A and panel C, group R) and the mean and SEM of the ratio of the fibrotic area of the rest of the kidney protomicrographs analyzed in 3–4 animals in each group (panel D). No significant difference in the fibrotic area was observed among the groups studied.

Table 1
General characteristics of animals.

	C (N = 10)	A (N = 8)	R (N = 10)
Initial weight (g)	209.1 ± 6.4	214.7 ± 2.2	203.2 ± 4.6
30-day weight (g)	198.4 ± 8.3	195.0 ± 13.5	188.0 ± 5.7
60-day weight (g)	194.7 ± 8.1	192.6 ± 14.4	196.4 ± 5.4
Initial glycaemia (mg/dL)	430.1 ± 34.5	407.5 ± 12.2	360.4 ± 17.3
60-day glycaemia (mg/dL)	532.6 ± 26.9	584.1 ± 6.9*	470.6 ± 27.7
60-day glycosuria (mg/24 h)	984.5 ± 21.5	983.8 ± 33.4	984.2 ± 29.3
60-day diuresis (mg/24 h)	93.7 ± 3.0	91.5 ± 6.1	89.8 ± 4.8

Data are reported as means ± SEM. C: diabetic spontaneously-hypertensive rats control; A: diabetic spontaneously-hypertensive rats treated with amlodipine 15 mg/kg/day for 30 days; R: diabetic spontaneously-hypertensive rats treated with ramipril 1 mg/kg/day for 30 days; *$p < 0.05$ *vs* R (one-way ANOVA and *post hoc* Tukey).

Discussion

The present study demonstrates that, despite similar BP reduction and no intervention to normalise glycaemia, the ramipril treatment (blocking the renin–angiotensin system) is, by far, more effective than the amlodipine treatment in reducing albumin, TGFβ1 urinary excretion as well as renal glucose transporters in an animal model of diabetic nephropathy.

Consistent with previous reports by our group and other authors (Balbinott et al., 2005; Davis et al., 2004; Schaan et al., 2005a), hypertension and hyperglycaemia were present in streptozotocin-treated SHRs. Consequently, this allowed us to study both the haemodynamic

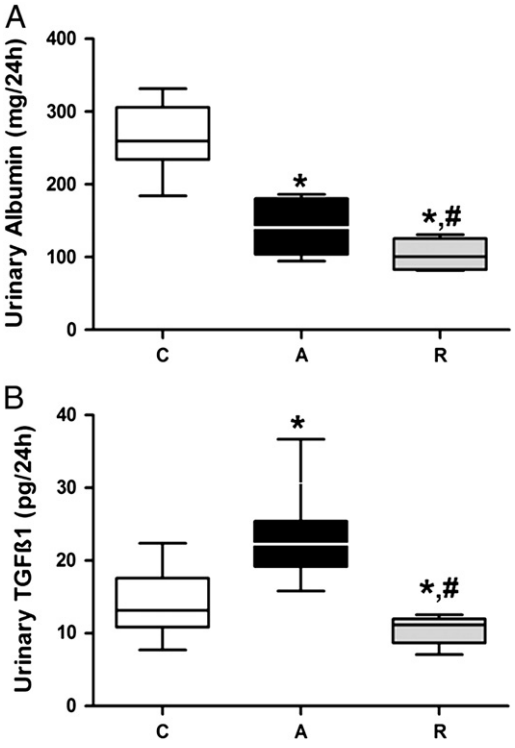

Fig. 2. Urinary albumin (panel A) and urinary TGFβ1 (panel B) in the groups studied. $^{*}p < 0.05$ *vs.* C, $\#p < 0.05$ *vs.* A. Data are reported as medians and p25th–75th. One way ANOVA; *post hoc* Tukey (data were log-transformed before analysis).

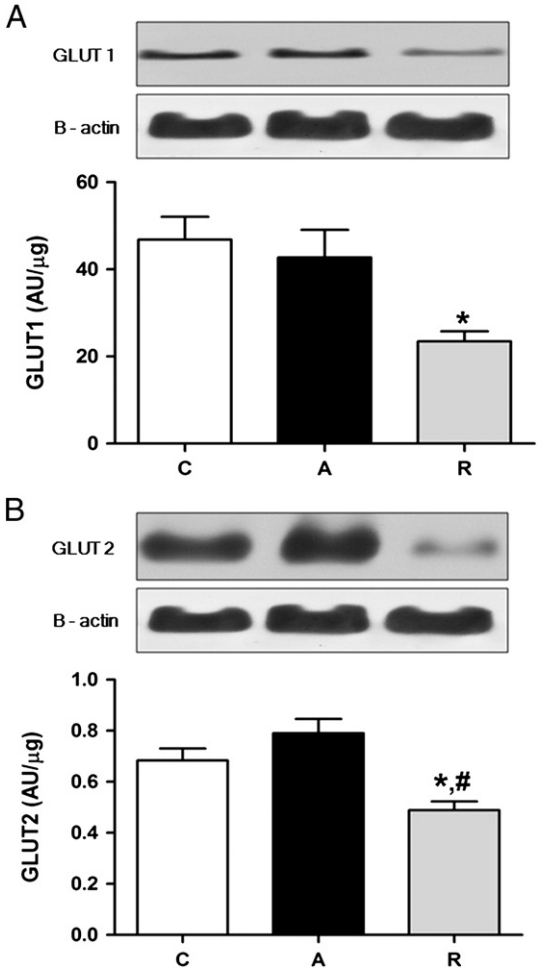

Fig. 3. Renal cortical GLUT 1 (panel A) and GLUT 2 (panel B) protein determined by Western blot analysis of samples in the groups studied. Loading controls are β-actin. $^{*}p < 0.05$ *vs.* C; $\#p < 0.05$ *vs.* A. Data are reported as means ± SEM. One way ANOVA; *post hoc* Tukey.

and metabolic derangements which diabetes can cause in renal function in this animal model. Hyperglycaemia and high levels of urinary glucose excretion remained stable throughout the study period (60 days), which was exactly our objective, to maintain hyperglycaemia constant and to change only BP levels during the experiment. Glycemias were higher in amlodipine-treated rats as compared to those treated with ramipril, but glycosurias, which provide a better global estimation of the hyperglycemic state throughout the day, were very similar among groups. Moreover, our objective of reducing BP to similar levels employing anti-hypertensive agents with different action mechanisms was achieved. An unexpected finding, however, was that amlodipine could reduce the renal angiotensin-converting enzyme activity, although not at the same magnitude observed in ramipril-treated rats. Although other authors have reported ACE expression inhibition by calcium channel blockade in aortic and cardiac tissues of rats with L-NAME hypertension (Kataoka et al., 2004; Toba et al., 2005), we report it in the kidney for the first time.

High levels of albuminuria, a functional feature of diabetic nephropathy, were effectively reduced by BP reduction with either agent used, but at a greater magnitude with ramipril. The effect caused by ramipril was not observed in a previous study (Souza et al., 2008), probably due to the shorter duration of treatment. In the present study, however, by using a longer period of treatment, ramipril-induced reduction in albuminuria was observed, as reported by other authors in long-term anti-hypertensive treatment in humans and rats (Parving et al., 1987; Zatz et al., 1986). In addition to the well-known effect of reduced BP on albuminuria reduction, anti-oxidative effects induced by renal ACE activity reduction could have contributed to these effects (Takao et al., 2011; Toba et al., 2005), as diabetic complications are primarily determined by excessive generation of reactive oxygen species (Brownlee, 2005).

Importantly, the high levels of urinary TGF-β1, a molecular marker of diabetic renal injury (Bertoluci et al., 1996), were even more elevated by the use of amlodipine, irrespective of reduced BP, whereas they were effectively reduced by ramipril. This particular effect of ramipril was previously shown in humans with diabetic nephropathy and non-diabetic renal proteinuric diseases (Bertoluci et al., 2006; Praga et al., 2003). The effect of amlodipine on increasing urinary TGF-β1 levels has also been reported previously (Praga et al., 2003). Furthermore, in the same animal model we used, Chen et al. (2002) reported that the renal protein expression of TGF-β1 measured by Western analysis can be prevented by ACE blocker cilazapril, but not by amlodipine, reinforcing local angiotensin II system involvement in the pathogenesis of renal injury in hypertensive diabetic rats by stimulating TGF-β1 expression. The powerful effect of ramipril on reducing renal angiotensin-converting enzyme activity, as compared to that of amlodipine, could account for the different effects of these drugs.

High levels of TGF-β1 presented by amlodipine-treated rats could cause overexpression of PAI-1, decreasing degradation and/or increasing production of extracellular matrix, leading to renal fibrosis. However, despite opposite changes observed in TGF-β1 of amlodipine- and ramipril-treated rats, fibrosis was not different between groups, showing that early changes in TGF-β1 were not yet accompanied by structural changes (fibrosis). Previous studies showed that Ang-II type 1 receptor (AT-1R) blockers could suppress the expression of TGF-β1 in the kidney of diabetic rats and attenuate renal lesions (Arozal et al., 2009; Lakshmanan et al., 2012). The different ways of blocking the renin–angiotensin system could account for the different results obtained in the present study. Moreover, no previous study showed the possible deleterious effect of amlodipine upon the functional marker of fibrosis, TGF-β1.

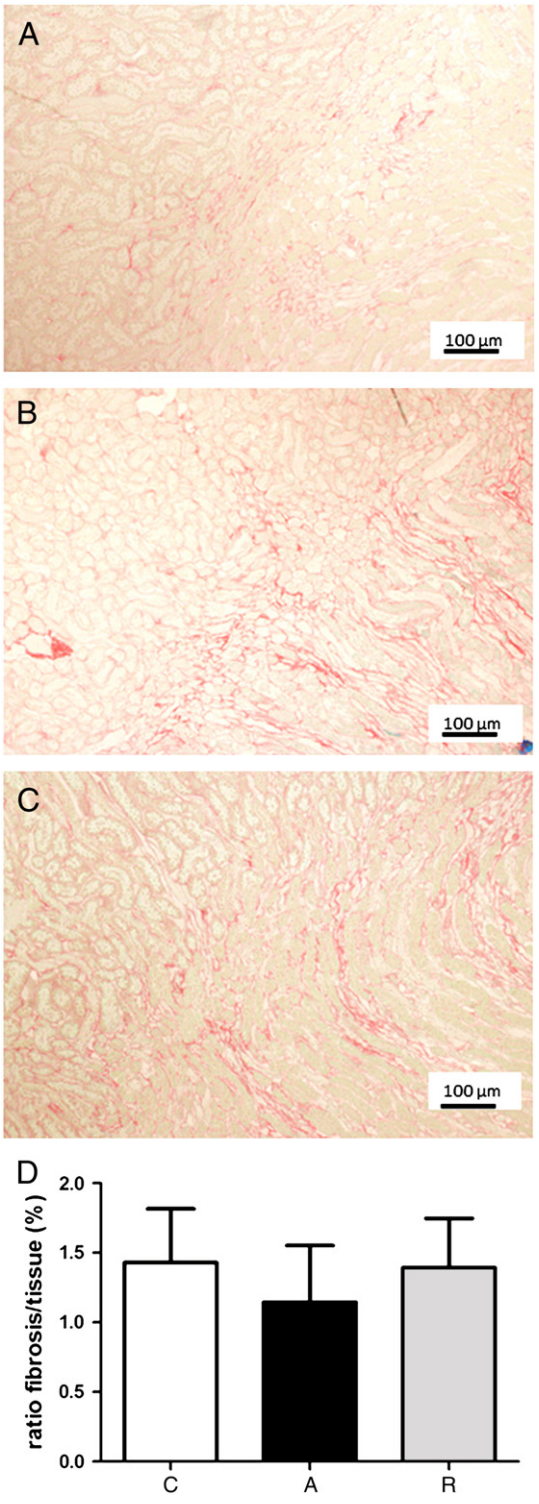

Fig. 4. Representative photomicrographs of Sirius red staining of C (panel A), A (panel B) and R (panel C) groups; collagens were stained as red. Data are reported as means ± SEM (Panel D). One way ANOVA; *post hoc* Tukey.

Mechanical stretch imposed by systemic hypertension on glomerular structures can itself promote overexpression of GLUT1, a mechanism that involves TGF-β1 signaling activation, suggesting that lowering BP levels, as observed previously (Souza et al., 2008), could be involved in GLUT1 expression reduction. In that report (Souza et al., 2008), we could not differentiate the effects of reducing BP and inhibiting the ACE with ramipril, as we are now showing with the present study design.

Now we are reporting a reduction of GLUT1 and GLUT2 content in rats treated with ramipril, which is expected to reduce the mesangial cell glucose disposal, contributing to reduction in the extracellular matrix production. Importantly, this effect on glucose transporter expression modulation does not seem to be only a consequence of BP lowering, but also related to mechanisms involving glucose transport. GLUT1 is the predominant glucose transporter in mesangial cells (Li et al., 2001), and GLUT1 overexpression in cultured mesangial cells induces increased glucose uptake and synthesis of extracellular matrix, collagen IV and fibronectin (Heilig et al., 1995). Additionally, changes in cortical renal GLUT1 expression have been associated with the development of diabetic nephropathy in vivo (Schaan et al., 2001, 2005a), and studies in transgenic mice and clinical investigations of the XbaI polymorphic alleles in intron 2 of the human SLC2A1 gene, which codifies the GLUT1 protein, have also associated changes in GLUT1 with nephropathy (Heilig et al., 2006). Non-diabetic transgenic mice, overexpressing glomerular GLUT1 protein predominantly in mesangial cells, develop microalbuminuria and glomerulosclerosis similar to that observed in diabetic mice (Wang et al., 2010). Finally, diabetic patients with the TT genotype at A–2841T in SLC2A1 gene (Hodgkinson et al., 2005), and diabetic or non-diabetic subjects with the SLC2A1 Enh2SNP1 homozygous AA genotype are at increased risk for kidney disease (Hsu et al., 2011). For these processes related to GLUT1 overexpression in mesangial cells, increased GLUT2 expression in tubular cells plays a fundamental role, contributing to increased interstitial glucose concentration. Whether the effect of ramipril on glucose transporters is able to nullify established damage or not will depend on the reversibility of these derangements, but it probably would decelerate the progression of nephropathy.

The present study was not designed to demonstrate molecular mechanisms involved in the observed effects of ramipril; however, we might speculate that the changes in glucose transporters not only could be a consequence of decreased levels of Ang-II, but also involve other ACE inhibition-induced effects such as increased bradykinin (Dendorfer et al., 2000; Soehnlein et al., 2005), potentiation of other kinins (Dendorfer et al., 2001), and high nitric oxide availability (Otto et al., 2006). Further studies should be designed to demonstrate the ACE inhibition-mediated mechanisms related to renal glucose transporter regulation.

Conclusions

The ramipril treatment (blocking the renin–angiotensin system) is, by far, more effective than the amlodipine treatment in reducing albumin, TGFβ1 urinary excretion as well as renal glucose transporters in an animal model of diabetic nephropathy. These results highlight that ACE inhibitor ramipril presents benefits not only associated with BP reduction in diabetic hypertensive individuals. Further studies should be performed to find out whether the beneficial effects of lowering BP with ramipril or other ACE inhibitors on renal glucose transporters expression are sustained for longer periods of time, and whether reducing glycaemia would have additive effects on the present findings.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

We thank Tatiana Sousa Cunha, Dulce Elena Casarini and Maristela Okamoto for their important role in the ACE activity determination and Western blotting analyses. The authors also thank Dr. Adauri Brezolin for final English revision of the manuscript.

This study was partially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à pesquisa do Rio Grande do Sul (FAPERGS), Fundo de Apoio à Pesquisa

do Instituto de Cardiologia (FAPICC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP, # 2012/04831-1).

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