Renal medullary angiotensinogen and hydrogen peroxide in Wistar and SHR diabetic rats

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Introduction

Hypertension and diabetes are major independent causes of kidney disease that frequently coexist worldwide, increasing the risk of renal damage and of progression to end-stage renal disease [1-3]. Current drug therapy is only capable of delaying the development and progression of renal damage [1, 2]. Overstimulation of renal renin-angiotensin system (RAS) [4] and increased production of reactive oxygen species (ROS) [5-7] are main pathophysiological mechanisms associated with the establishment and progression of kidney injury that occurs independently in diabetes and in hypertension. The interplay between RAS and ROS [8, 9] settles a vicious cycle that contributes to perpetuate renal damage.

The development of end-stage renal disease is strongly associated with renal medullary dysfunction. Oxidative stress contributes to disrupt medullary homeostasis which leads to damage [10, 11]. Previous reports suggest a close relation between medullary hydrogen peroxide (H_2O_2) production and the urinary angiotensinogen (AGT) excretion that may be involved in medullary dysfunction [9]. The association when both hypertension and diabetes are present is currently not established.

In the present study, we hypothesized that induction of diabetes in hypertensive animals would exacerbate the renal damage that would occur in normotensive animals, namely that operating through H_2O_2 and AGT production.

Objective

Study of renal AGT and H_2O_2 production in diabetes associated with hypertension.

Method/Approach

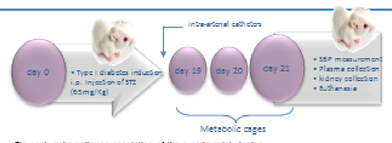

Figure 1: schematic representation of the experimental design.

Animals. Male Wistar (11-12 weeks) and SHR (12 weeks) were used. On day 0, diabetes was induced by an i.p. injection of STZ (65 mg/kg in citrate buffer, pH 7,4). Control rats recived i.p. injection of vehicle.

Systolic blood pressure (SBP). SBP measurements were performed on day 21 in conscious unrestrained animals.

Metabolic study. At the end of the study, animals were housed in metabolic cages. Body weight, water and food intakes were monitored. Twenty-four hour urine excretion was quantified and 24h-urine samples were kept frozen until assayed, as well as kidney samples.

Quantifications. Glucose and creatinine concentrations were determined in urine and plasma samples by the glucose oxidase method and the colorimetric Jaffé method, respectively, and urinary total protein concentration was determined using pyrogallol red. These assays were performed using a Cobas Mira Plus analyser. Glomerular filtration rate (GFR) was calculated using the formula GFR = U * V / P, where U and P denote creatinine concentration in the urine and plasma samples, respectively, and V denotes the urine flow rate (ml/min). H_2O_2, 8-Isoprostane and AGT were quantified using commercial kits according to the protocols provided by the manufacturers (Amplex Red Hydrogen Peroxide Assay kit, Urinary Isoprostane ELISA Kit and Rat Total Angiotensinogen Assay Kit, respectively). AGT protein expression was performed by Western Blot (AGT Abbiotec 250551; 1:200) using total lysate of renal medullary or cortical homogenates Quantification of thiobarbituric acid reactive substances (TBARS) was performed according to the method of Ohkawa.

Data analysis. Statistical interactions between groups were evaluated by two-way ANOVA followed by Tukey's test. Values are means±SEM. $p<0.05$ was considered statistically significant. Pearson's single regression analysis was used to estimate correlation between parametric data.

Results

Systolic Blood Pressure

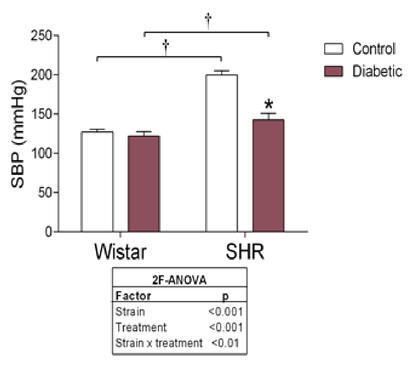

Figure 2. Systolic blood pressure (SBP; mmHg) of Wistar and SHR control and diabetic rats. Results expressed as mean±S.E.M., * $p<0.05$ vs corresponding control group; † $p<0.05$ vs corresponding Wistar group.

Plasma and Urinary AGT

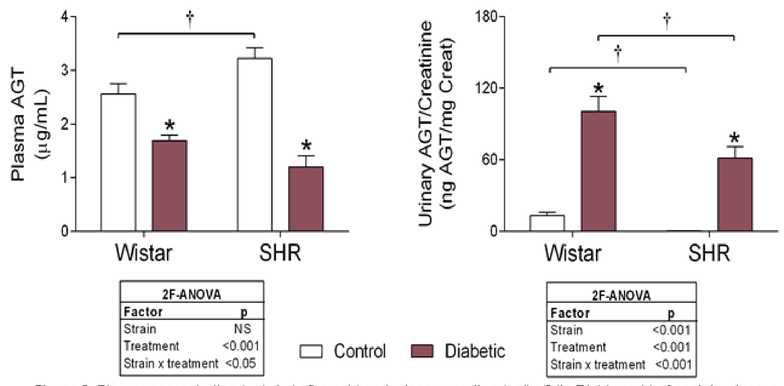

Figure 3. Plasma concentration (µg/mL; Left graph) and urinary excretion (ng/kg/24h; Right graph) of angiotensinogen (AGT) of Wistar and spontaneously hypertensive (SHR) control and diabetic rats. Results expressed as mean±S.E.M.; * $p<0.05$ vs corresponding control group; † $p<0.05$ vs corresponding Wistar group.

Renal AGT Expression

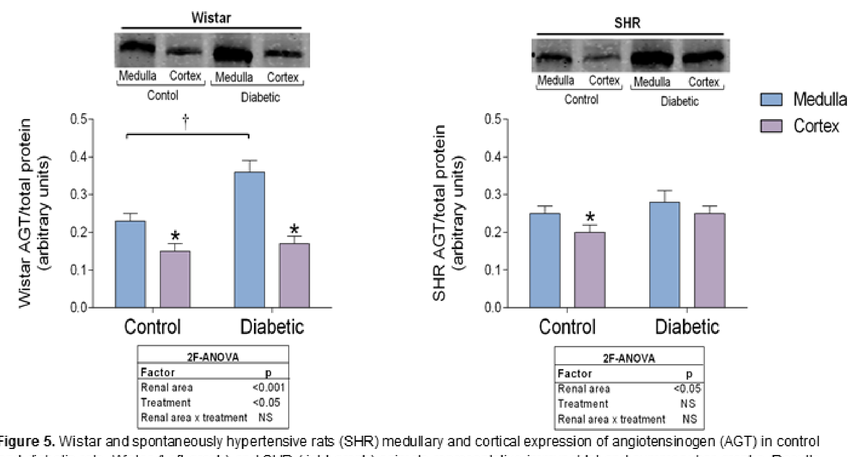

Figure 5. Wistar and spontaneously hypertensive rats (SHR) medullary and cortical expression of angiotensinogen (AGT) in control and diabetic rats, Wistar (Left graph) and SHR (right graph) animals representative immunoblot and summary bar graphs. Results expressed as mean±S.E.M; * $p<0.05$ vs corresponding renal medulla; † $p<0.05$ vs corresponding control renal area.

Metabolic and Renal Function Parameters

Table 1. Metabolic and renal function parameters of Wistar and SHR rats in the respective control and diabetic groups.

	P-glucose (mg/dL)	BW (g)	Food intake (g/24h)	Water intake (mL/24h)	U-excretion (mL/24h)	U-glucose (g/kg/24h)	U-proteins (mg/kg/24h)	GFR (mL/min)
Wistar-control	190.0 ± 9.9	306.3 ± 7.4	20.3 ± 1.2	26.2 ± 2.2	16.0 ± 2.6	0.0 ± 0.0	48.2 ± 4.0	1.4 ± 0.1
Wistar-diabetic	596.3 ± 38.3*	223.3 ± 5.7*	38.2 ± 1.4*	183.0 ± 14.1*	200.1 ± 10.1*	30.5 ± 2.6*	189.7 ± 22.8*	2.7 ± 0.2*
SHR-control	165.2 ± 8.0	279.2 ± 7.4†	13.9 ± 2.1†	25.1 ± 4.0	7.0 ± 0.8	0.1 ± 0.0	62.0 ± 3.7	1.4 ± 0.1
SHR-diabetic	397.6 ± 42.5*†	175.8 ± 8.3*†	25.1 ± 1.2*†	125.3 ± 8.3*†	104.0 ± 8.9*†	22.4 ± 2.7*	201.4 ± 21.6*	2.2 ± 0.3*
Strain	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	NS	NS	<0.05
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Interaction	< 0.01	NS	<0.05	<0.05	<0.001	NS	NS	NS

Abbreviations: P-plasma; BW-body weight; U-urine; GFR-glomerular filtration rate.
Results expressed as mean±S.E.M. *denotes $p<0.05$ *versus* corresponding control group; †denotes $p<0.05$ *versus* corresponding Wistar group.

Renal H_2O_2 Production

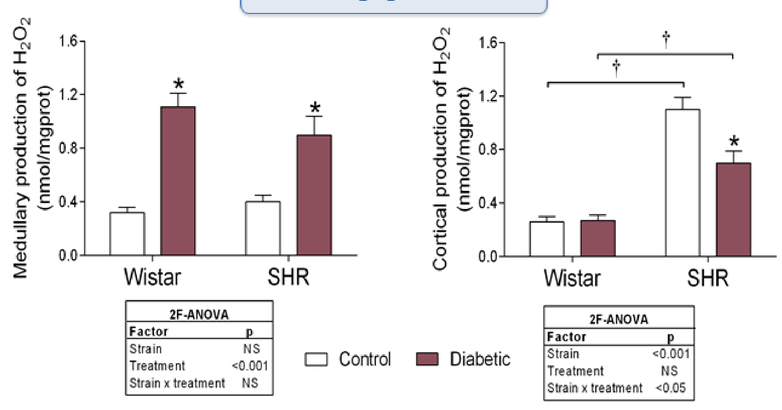

Figure 4. Renal medullary (Left graph) and cortical production (Right graph) of hydrogen peroxide (H_2O_2; nmol/mgprot) of Wistar and spontaneously hypertensive (SHR) control and diabetic rats. Results expressed as mean±S.E.M.; * $p<0.05$ vs corresponding control group; † $p<0.05$ vs corresponding Wistar group.

Urinary Oxidative Stress Parameters

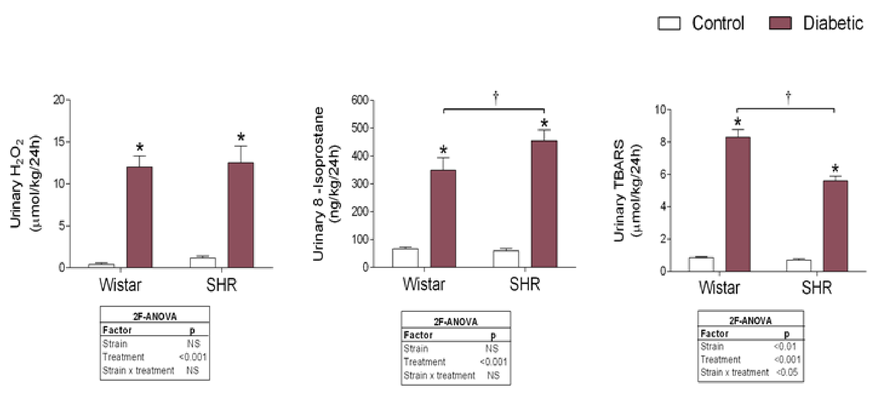

Figure 6. Urinary excretion of hydrogen peroxide (H_2O_2; µmol/kg/24h; Left graph), 8-isoprostane (ng/kg/24h; Center graph) and thiobarbituric acid reactive substances (TBARS; µmol/kg/24h; Right graph) of Wistar and spontaneously hypertensive (SHR) control and diabetic rats. Results expressed as mean±S.E.M; * $p<0.05$ vs corresponding control group; † $p<0.05$ vs corresponding Wistar group.

Correlation analysis

In Wistar and SHR animals, Pearson's single regression analysis revealed:

- positive correlation between renal medullary production of H_2O_2 and plasma glucose concentration
  Wistar: $r^2=0.5135$, $r=0.7166$, $p<0.05$, $n=10$
  SHR: $r^2=0.6239$, $r=0.7899$, $p<0.05$, $n=14$
- positive correlation between renal medullary production of H_2O_2 and urinary excretion of AGT
  Wistar: $r^2=0.6945$, $r=0.8333$, $p<0.05$, $n=6$
  SHR: $r^2=0.6933$, $r=0.8326$, $p<0.01$ $n=12$
- negative correlation between urinary excretion of AGT and plasma AGT concentration
  Wistar: $r^2=0.7428$, $r=-0.8619$, $p<0.01$, $n=6$
  SHR: $r^2=0.6526$, $r=-0.8078$, $p<0.001$, $n=15$

Normoglycemic Wistar and SHR animals showed similar renal function and metabolism along with parallel alterations in proteinuria and GFR following diabetes induction (Table 1). Oxidative status, evaluated by the quantification of urinary H_2O_2, isoprostane and TBARS, was significantly and comparably higher in diabetic groups of both strains (Figure 6).

The urinary AGT excretion was increased in diabetic rats of both strains (Figure 3). This was paralleled by an increase in the production of medullary H_2O_2 (Figure 4), and a positive correlation was observed between both parametrs. Concerning the renal expression of AGT, we observed a higher expression of AGT in the renal medulla when compared to the renal cortex in the Wistar-control, Wistar–diabetic and SHR-control groups (Figure 5). This pattern of AGT distribution is not explained by the results on medullary or cortical H_2O_2 production (figure 4) or by those on urinary AGT excretion (figure 3). The techniques used to measure AGT in the urine and renal tissue (ELISA and Western blot) identify the same molecules, but the origin of the AGT they measure is different. The ELISA kit was used to measure urinary AGT excretion, which reflects its *de novo* production in the S3 segment of the proximal tubule [12]. The AGT expression quantified by Western Blot reflects the renal interstitial/intracellular AGT protein content. Taking the present results, it is plausible that medullary H_2O_2 drives *de novo* production of tubular AGT, that is independent of the renal tissue AGT.

SHR-control animals had higher SBP than Wistar-control animals (Figure 2). Diabetes induction was associated with a decrease in SBP, only in SHR animals (Figure 2). Also, we have observed a negative correlation between urinary excretion of AGT and plasma AGT concentration in both strains. Although further experiments need to be performed in order to elucidate the mechanism(s) involved, plasma and renal-derived AGT may be synchronized and influence SBP.

Conclusions

Medullary H_2O_2 seems to be associated with tubular AGT production but not with renal tissue AGT expression. Also, hyperglycemia-induced increase in medullary H_2O_2 production and urinary AGT excretion is similar in normotensive and hypertensive animals. Furthermore, plasma and renal-derived AGT may be synchronized and influence SBP.

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