Oxidative Stress Unify the Pathophysiological Mechanism of Experimental Hypertension and Diabetes of Spontaneously Hypertensive Stroke-Prone Rats

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Research Article

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ABSTRACT

This study has as an attempt to identify both: oxidative stress and a possible kidney injury among rats with severe hypertension and diabetes. Twenty-four, 20 wk age, kept in baseline for ten days, were subdivided in four groups of six animals each: SHRSP, SHRSPDb, WKY, WKYDb. Systolic pressure was measured by plethysmography. Blood glucose by Accu-Check glucometer (Roche), Blood Urea Nitrogen, serum creatinine, and 24hours urinary albumin followed the routine laboratory methodology. Blood homocysteine was determined by HPLC. The data was statistically analyzed by ANOVA and $P<0.05$ was considered significant. Afterwards, the rats under deep coma were sacrificed and the kidney removed for histophatological analysis. Those hypertensive rats presented the highest levels of blood urea nitrogen levels: SHRSP (40 ± 3 mg/dl) and SHRSPDb (57± 5 mg/dl) versus WKY (17 ± 2 mg/dl) and WKYDb (22 ± 3.5 mg/dl). At the same way, the serum creatinine levels were significantly increased in hypertensive rats: SHRSP (1.20 ± 0.02 mg/dl), SHRSP (1.27± 0.04 mg/dl) versus WKY (0.53 ± 0.02 mg/dl) and WKYDb (0.49 ± 0.01 mg/dl). The 24 hours urinary albumin was also markedly increased in SHRSP strain: SHRSP (128 ± 1.2 mg/24 h) and SHRSPDb (125 ± 0.6 mg/24 h) versus WKY (15 ± 0.02 mg/24 h) and WKYDb (14.8 ± 0.01 mg/24 h).The Blood Homocysteine levels were significantly increased in the SHRSP strain: SHRSP (6.51 ± 0.40 µmol/l) and SHRSPDb (7.32 ± 0.61 µmol/l) as compared to those normotensive ones: WKY(3.80 ± 0.39 µmol/l) and WKYDb (5.20 ± 0.21 µmol/l). The histophatological assay confirmed a significant association between hypertension and renal damage, since the number of hyalinized glomeruli and vessel were higher in those hypertensive rats. However, the presence of an increased blood homocysteine levels in both strains suggests that an oxidative stress was common feature to the hypertension and diabetes.

INTRODUCTION

Hypertension is present in more than 80% of patients with chronic kidney disease and contributes to progression of Kidney disease toward end stage renal disease (ESDR) as well as to cardiovascular events and stroke [1]. There is a peculiar relationship between the Kidney and arterial blood pressure while renal dysfunction causes an increase in arterial blood pressure and this accelerates the loss of renal function [2]. In fact, the progression of hypertension and the presence of kidney injury become worse when in presence of diabetes. In fact, the advanced glycation end products in diabetes might be responsible for the nerve damage [3]. The coexistence of these diseases is clearly involved in the morbidity and mortality.

Therefore, the present study has as main goal to identify while kidney function is compromised in spontaneously hypertensive stroke-prone rats (SHRSP), a known animal model for study severe hypertension and stroke, and to explore a possible common mechanism involved in the pathogenesis of hypertension and diabetes as well.

METHODS

Animals and Groups

After an adaptation period of two weeks, twenty-four adult (20 WK age)male rats were divided into four groups with six animals each :Group I- Spontaneously Hypertensive Stroke-Prone Rats (SHRSP),Group II- SRHSP Diabetes-induced (SHRSPDb), Group III- Wistar-Kyoto normotensive rats (WKY) and Group IV- Wistar-Kyoto normotensive Diabetes-induced (WKYDb). The animals were maintained in metabolic cages in bioterium with controlled temperature (21 ± 2°C), humidity-controlled (60 ± 10%), and 12 dark/light cycle (artificial lights, 7 a.m-7 p.m), air exhaustion cycle (15 min/h) receiving water and pellets food (Nuvilab, Nuvital, Brazil) ad libitum. All the procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication no 85-23, revised 1996) and were approved by the Ethics Committee for Animal Use(CEUA) of Federal University of State of Rio de Janeiro,N°2016.2.

Diabetes Model

Diabetes was induced by streptozotocin (S-0130 Sigma, St.Louis, MO), dissolved in citrate buffer 0.1 M, pH 4.4, at single dose of 40 mg/kg of body weight, intraperitoneally. The blood glucose was measured on animals in fasting using the glucosimeter Accu-Check Advantage (Roche) at 72 hours after STZ and once a week. The Diabetes model was considered done when the rats reached the minimum blood glucose levels of 300 mg/dl, which occurred at 28 days after STZ.

Systolic Blood Pressure

The systolic blood pressure was verified in the morning, twice a week, using the noninvasive method of tail-cuff plethysmography in conscious rats (Letica LE 5100, Panlab).

Biochemical Assay

All groups were submitted to blood collection in order to determine: blood urea nitrogen (BUN) and serum creatinine and to 24 urine output to determine the urinary albumin, using routine laboratory methodology. Blood homocysteine was determined by HPLC.

Histopathological Analysis

After the experiment period, rats of all groups were in deep coma induced by administration of barbiturates (thiopental sodium) intraperitoneally at dose 60 mg/kg body weight. The kidneys were removed and stored in formaldehyde solution 10%, thereafter the slices were prepared for microscopic analysis, being carried cut 5 μm thick, the Gung RM 2025 microtome (Leica, Nussloch, Germany). The specimens were stained with hematoxylin-eosin. Morphometric analysis was performed using an optical light microscope (Zeiss Axioplan, Zeiss, Germany) under 10X, 40X, 60X. The number of hyalinization of glomeruli and vessels were observed in cross section samples from 50 fields per slide at high magnification/400X.

Statistical Analysis

The data were presented as mean ± standard error, and applied the ANOVA test for comparison of the four groups. $P<0.05$ was considered statistically significant.

RESULTS

The determination of systolic blood pressure confirmed the higher levels in SHRSP strain which presented as mean values of 225 ± 2.5 mmHg versus 140 ± 3.7 mmHg presented by WKY strain. There were a significant increased BUN and serum creatinine levels in SHRSP rats, twice as those found in WKY rats **(Figures 1 and 2)**.

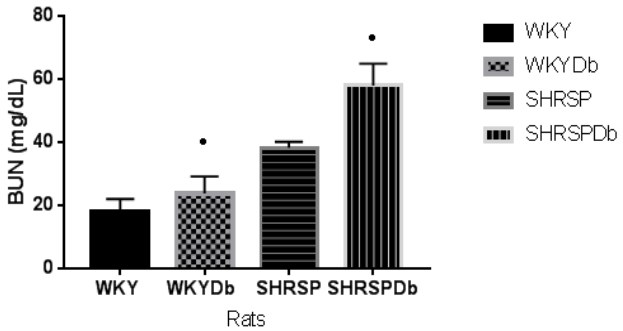

Figure 1. The data represent mean ± SE of Blood Urea Nitrogen level from six animals per group. $P<0.05$ was considered significant between SHRSP and WKY rats and between SHRSPDb and WKYDb rats.

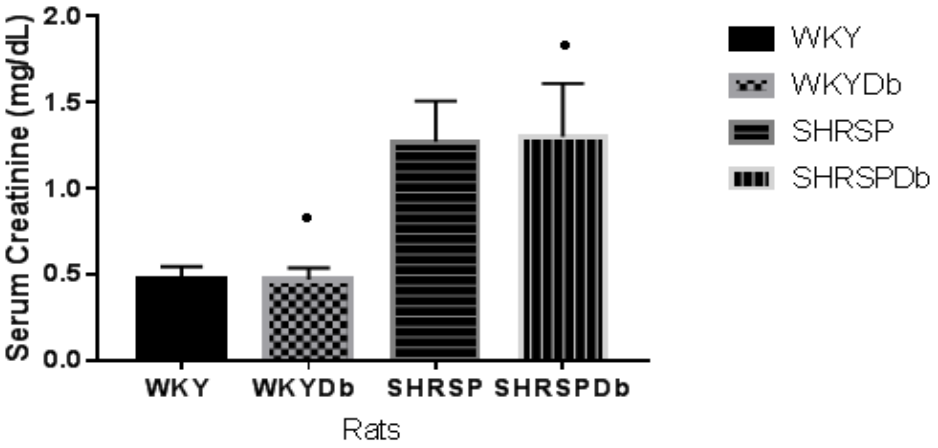

Figure 2. The data represent mean ± SE of Serum Creatinine level from six animals per group. P<0.05 was considered significant between SHRSP and WKY rats and between SHRSPDb and WKYDb rats.

At the same way, the urinary albumin was markedly elevated at SHRSP strain in comparison to normotensive Wistar-Kyoto rats **(Figure 3)**.

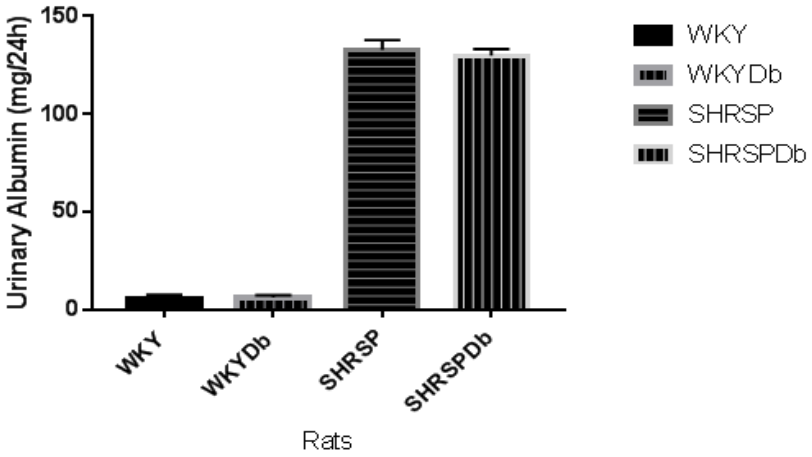

Figure 3. The data represent mean ± SE of Urinary Albumin level from six animals per group. P<0.05 was considered significant between SHRSP and WKY rats and between SHRSPDb and WKYDb rats.

There was also a significant increase of homocysteine blood levels at SHRSP rats which was more accentuated in those diabetes SHRSP rats. At the same way, an elevated levels of blood homocysteine was found in those WKYDb rats **(Figure 4)**.

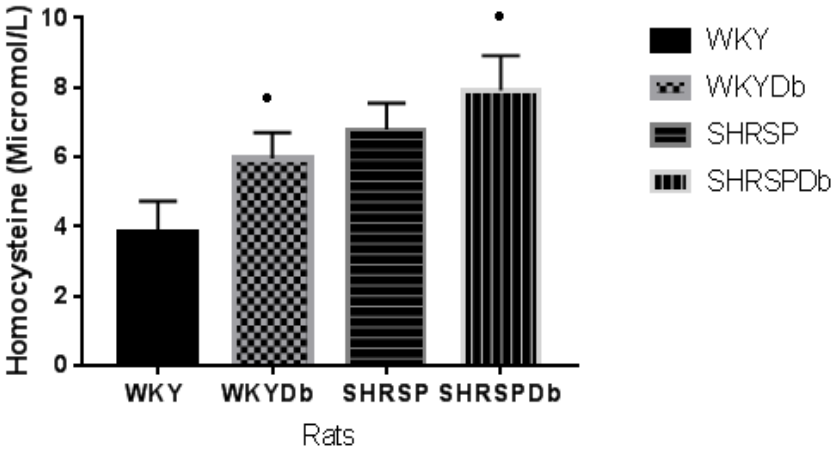

Figure 4. The data represent mean±SE of blood Homocysteine levels of six animals per group. The significance was considered as P<0.05

Regarding to kidney histophatological assays, there were a significant decrease of hyalinization of the glomeruli and renal vessels of normotensive strain compared to hypertensive groups independent of diabetes **(Figures 5 and 6)**.

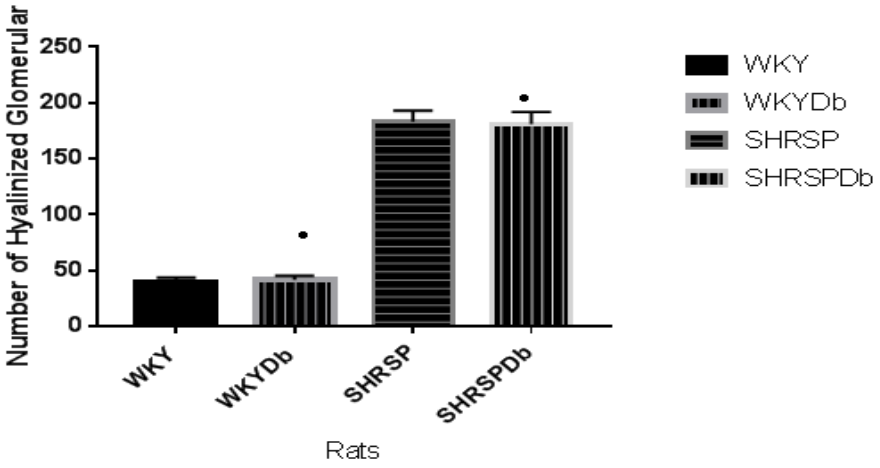

Figure 5. The data represent mean ± SE of Hyalinized Glomeruli of six animals per group. P<0.05 was considered significant and it was found between SHRSP versus WKY and between SHRSPDb versus WKYDb rats.

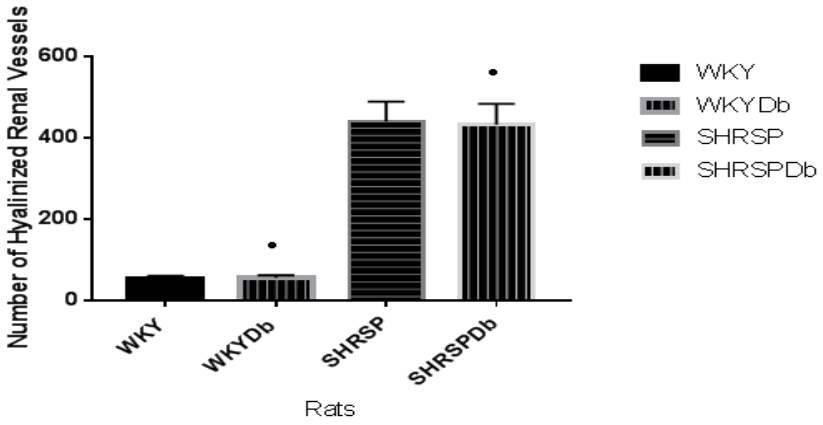

Figure 6. The data represent mean ± SE of Hyalinized Renal Vessels of six animals per group. The significance level of P<0.05 was found between SHRSP versus WKY and between SHRSPDb rats versus WKYDb rats.

DISCUSSION

Although novel biomarkers are in practical use, serum creatinine an indicator of glomerular filtration rate still the most frequently used biomarker of renal function, despite its known limitations. Here, together with BUN measurement, and the histophatological findings, it was possible to confirm the kidney injury as consequence of severe hypertension.

Hyperhomocysteinemia was also observed in all hypertensive rats which is an agreement with previous studies from our group, that demonstrated the high levels of homocysteine and others markers of oxidative stress, such as malondialdehyde in SHRSP rats [4].

Although diabetes normotensive rats presented an increased homocysteine blood levels than those controls rats, the kidney injury, was not observed in those normotensive diabetes rats in comparison to hypertensive ones.

On the other hand, in the presence of longer experiment such differences might be more evident. According to Hong JH et al. a persistent and chronic hyperglycemia, could deplete the activity of the antioxidant defense system promoting the generation of free radicals and more pronounced organ damage could be observed [5].

The renal histopathology revealed an important tissue damage in samples of all SHRSP rats including those diabetes. In fact, mesangial cell proliferation and glomerular sclerosis has been demonstrated in different experimental models of hypertension [6]. In addition, hyperhomocysteinemia induces to DNA hypermethylation, leading to an abnormal metabolism in the kidney and damage of glomerulo tissue [7].

CONCLUSION

In sum, this study confirms the importance of SHRSP rats as model for study of both: severe hypertension and kidney injury, besides those features linked to the neurological disturbances. At the same time, the blood homocysteine, an important known biochemical marker of an oxidative stress was closely linked to both hypertension and diabetes in the present study, suggesting a unique pathophysiological mechanism.

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