The Diabetic Nephropathy and the Development of Hypertension in Rats

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The present study was designed to examine the development of hypertension in diabetic rats treated with streptozotocin (STZ, 1mg/g bw). The rats were studied at 3, 6, 9, 12 and 15 weeks. From the third week the rats were divided in diabetic rats according their glycemas and controls, along 15 weeks. After the third week a group of rats showed increased urinary protein excretion (93, 134, 155 and 191\%) compared to controls. In this group of rats the urinary kallikrein excretion was lower than control and the systolic blood pressure became significantly elevated between 3 and 6 weeks and persisted up to 15 weeks. On the other hand a group of diabetic rats were not hypertensive with urinary protein excretion similar to controls and urinary kallikrein lower compared to control but significantly higher compared diabetic hypertensive rats. These data suggest that the association of progressive diabetic nephropathy with abnormal endothelium-dependent vasodilation may produce a high prevalence of hypertensive diabetes.

Keywords: Diabetes; Hypertension; Proteinuria; Kallikrein

INTRODUCTION

Renal kallikrein is a serine protease located in the distal cortical nephron.\cite{1} By its action on kininogen substrate it generates potent vasoactive kinins. Several observations link this enzyme and its product to the regulation of hemodynamic and ion transporting processes. Diabetes mellitus and hypertension are common chronic conditions which frequently coexist. According to Viberti \textit{et al.}\cite{2} the progression of diabetic nephropathy appears to be closely related to blood pressure elevation. Several studies show that the measure of urinary protein loss correlates with the rate of renal function deterioration. Early morphologic lesions of diabetic nephropathy develop in practically all IDDM within a few years after the onset of their metabolic abnormality.\cite{3} On the other hand essential hypertension accounts for the majority of cases of hypertension in the diabetic population. Extensive evidence indicates an association of kallikrein-kinin system with blood pressure regulation. In the present study, we investigated whether the progressive increase of proteinuria and the decreased urinary kallikrein excretion may contribute to the hypertension in the diabetic state.

MATERIALS AND METHODS

Neonatal male rats (2 days old) were used in his study. They were kept in a temperature controlled environment with standard laboratory food and water freely available. Diabetes was induced by a single subcutaneous injection of...
streptozotocin (STZ), 1mg/Kg bw dissolved in citrate buffer (pH 4.5). Control rats of the same age were injected with buffer alone. Blood samples were collected from the tail vein in subsequent periodic collections. Rats were housed two to three per cage, and had access to water and food ad libitum. Blood was collected from the tail vein and urinary samples were collected housing the animals in metabolic cages during 24h. Systolic blood pressure (SBP) was measured at different times with a pneumatic pulse transducer and occluding tail cuff (Grass Mod. 79 Poligraph, Grass Inst. Mass, USA). Rats were placed in restrainers at 28–30°C and after 1h, 4–6 determinations of SBP were made and average.

Analytical Methods

Diabetes was confirmed by plasma glucose levels measured after STZ injection and at 21, 42, 63, 85, 105 days. Urinary protein was measured according to the procedure of Lowry et al. and kallikrein was assayed as previously described. These parameters were measured over a period of 15 weeks. Glomerular Filtration rate (GFR) was measured according to Henry et al. Creatinine was measured with a Wiener kit reagent (Wiener Arg. SA).

Chemicals

STZ was purchased from Sigma Chemical Co., St. Louis, Mo. It was dissolved in 0.02M citrate buffer, pH 4.5 and immediately injected intraperitoneal. The kallikrein assay was performed using the synthetic chromogenic substrate H-D-Val-Leu-Arg-pNA (S2266) from Kabi, Stockholm, Sweden. The para-nitroanilide (pNA) released by enzymatic reaction on the substrate was measured calorimetrically to determine kallikrein activity.

Statistical Analysis

Data are expressed as mean +/− SEM. Differences were assessed by ANOVA. Intergroup differences were analyzed by Student's test for unpaired data. Differences were considered significant at a level of P < 0.05.

RESULTS

Three weeks after the initial STZ injection diabetic hypertensive rats (DH) showed significantly lower body weight compared to control rats. The average weight gain in DH rats was 2.33+/−0.27 vs. 3.14+/−0.31g/day. No differences were observed in diabetic normotensive (DN) compared to controls. After the 15th weeks these differences persisted and the average was 2.48+/−0.3 vs. 3.21+/−0.14g/day. The kidney/body weight ratio also, was significantly different at the 3rd and 15th weeks in DH rats. GFR was observed significantly reduced in DH rats compared to controls (Tab. I).

When the diabetic and control rats were studied at different periods of time the following results were obtained:

Blood glucose levels: Glucose steadily increased in one group of diabetic animals over time from

| TABLE I Different parameters of the control and diabetic rats |
|-------------------------------------------------------------|
| 3rd week | 15 weeks |
| Control | DN | DH | Control | DN | DH |
| Body weight (g) | 330+/−16 | 302+/−10 | 245+/−17* | 341+/−14 | 312+/−11 |
| Kidney weight (g) | 3.2+/−0.02 | 2.8+/−0.5 | 2.9+/−0.4 | 3.1+/−0.4 | 3.8+/−0.6 |
| Body weight ratio % | 0.97+/−0.04 | 0.93+/−0.03 | 1.18+/−0.08* | 0.90+/−0.02 | 1.19+/−0.04 |
| GFR ml/min | 0.37+/−0.05 | 0.50+/−0.04* | 0.38+/−0.06 | 0.39+/−0.05 | 0.45+/−0.02 |

DN: Diabetic Normotensive; DH: Diabetic Hypertensive.

Data are +/− DE *P<0.01 Diabetes vs. Control.

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the 3rd week to 15th weeks (30, 48, 47, 46 and 49% respectively). On the other hand other group of diabetic rats showed 8, 25, 30 and 30% increased blood glucose over time (Fig. 1).

**Urinary protein excretion:** Urinary protein excretion was observed progressively increased over time in one group of diabetic rats (93, 134, 155, 199% DH) started from the 3rd week. In the DN rats the increase of urinary protein secretion was 25, 45, 50 and 57 from the 6th week (Fig. 1).

**Total Kallikrein excretion:** Kallikrein excretion was observed progressively decreased in DH rats from the 3rd to 15th weeks compared to controls. However in DN rats the urinary kallikrein excretion was significantly different from the 6th week compared to control (Fig. 3).

**DISCUSSION**

The present study has clearly shown that increased urinary protein excretion and lower
kallikrein secretion is, probably one of the possible mechanisms to produce diabetic hypertension. Marre et al.\cite{8} suggested that microalbuminuria in some hypertensive NIDDM patients can be explained by the association between essential hypertension and NIDDM. Diagnosis of clinical nephropathy, however, is made by the appearance of persistent proteinuria which inaugurates a phase leading to an end-stage renal disease (ESDR). Thus hypertension is often associated with diabetic nephropathy.\cite{9} Ours study examine the mechanisms responsible for the abnormalities in the renal function associated with the diabetic hypertension. We found that diabetes can produce some changes in the progressive increase of urinary protein excretion and reduced kallikrein secretion when blood glucose is persistently higher over time. Even though other mechanisms may contribute to reduce tubuloglomerular feedback and hyperfiltration in diabetes mellitus, stimulation of cotransporter by hSGK is likely to participate in the generation of diabetic hyperfiltration. Beyond that, stimulation of epithelial Na\textsuperscript{+} channel and cotransporter is expected to induce Na\textsuperscript{+} retention and thus favour the development of diabetic hypertensive disease.\cite{10} In Fact, the prevalence of hypertension in this case correlates with the renal functional abnormalities and the duration of diabetes.\cite{11} According to Christlieb\cite{12} essential hypertension accounts for the majority of cases of hypertension in the diabetic population, and the pathogenesis of essential hypertension in diabetic patients is considered to be similar to that in non diabetic patients.\cite{13} Furthermore an study by Knowler et al.\cite{14} on Pima Indians showed that prediabetic elevated blood pressure occurred at higher rate (36%) in those patients with increased proteinuria and nephropathy than those with low or without proteinuria and nephropathy (12%) Interestingly diabetic rats with moderate hyperglycemia, low proteinuria and low kallikrein from the 9th week did not develop hypertension. The mechanisms trough which urinary protein excretion did not rise and kallikrein excretion was low in this group of DN rats is not completely clear. Previous studies have indicate that diabetic patients in poor control of their prior glycemia (HbA 1c 11%) had 177% of urinary protein excretion compared to controls, whereas diabetic patients with HbA 1c 11% showed less of 100% of urinary protein excretion. These results showed also that kallikrein secretion rate was correlated with the HbA 1c level.\cite{15} The magnitude of the diabetes state produces an impairment of rat renal kallikrein synthesis with a decreased renal tissue and urinary kallikrein secretion as it was demonstrated by Jaffa et al.\cite{16} in diabetic rats. On the other hand insulin treatment increased kallikrein mRNA levels, suggesting that diabetes suppresses kallikrein gene expression. Severely hyperglycemic diabetic rats with normal GFR had lower renal excretion of active kallikrein than control animals, whereas moderately hyperglycemia diabetic rats exhibited higher urinary levels of the enzyme.\cite{17} According with our results in the group of rats with diabetic nephropathy the proteinuria was significant increased over the time with a correlation with decreased kallikrein. Taken together the data suggest that there is a defect in the expression and transcription of kallikrein in the diabetic kidney as it was postulated in diabetic rats by Jaffa et al.\cite{16} Diabetic nephropathy produces structural abnormalities as hypertrophy of kidney, increase in the thickness of glomerular basement membranes, accumulation of extracellular matriz in the glomerulus, tubular atrophy and interstitial fibrosis.\cite{17} It is relevant to point out that functional alterations of diabetic nephropathy produces an early proteinuria and systemic hypertension.\cite{18} In this regard, in human and animal diabetic models TGF-beta mRNA and protein levels are significantly increased in the glomeruli and tubulointerstitial.\cite{17} On the other hand the deleterious effect of proteinuria on the progression of renal disease may be mediated via TGF-beta.\cite{19} The suggestion that severely hyperglycemic diabetic rats with increased proteinuria and low kallikrein secretion shows hypertension is further supported by studies that shows that
endothelium function is abnormal in diabetic rats. In conclusion, the present study has found that in long-term experimental diabetes the progressive increase of urinary protein secretion in the early state of diabetes and low kallikrein excretion is one of the major pathways responsible of diabetic hypertension.

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