Prolactin receptor mRNA expression in experimental diabetic nephropathy: Relationship with urinary albumin excretion

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Abstract

OBJECTIVES: Disturbances of prolactin secretion, peptide hormone produced by the pituitary gland, occur in both the chronic renal failure and in diabetes mellitus. So far, the role that prolactin play in the pathology of diabetic nephropathy progression is still unclear. Therefore, the present study was intended to examine whether the renal mRNA expression of prolactin receptor (PRL-R) is altered in experimental diabetes, and how such changes related to the development of albuminuria.

METHODS: Adult female wistar rats were divided into two groups (n=6/group): control (non-diabetic) and diabetic. Diabetes was induced by a single dose injection of 55 mg/ kg streptozotocin. After ten weeks, renal mRNA expressions of both short and long forms of PRL-R were evaluated by real-time polymerase chain reaction.

RESULTS: Diabetes was associated with increases in urine albumin excretion (UAE), kidney weight/body weight ratio, serum prolactin and the mRNA expression of both short and long forms of PRL-R were evaluated by real-time polymerase chain reaction. Furthermore, UAE was significantly correlated with serum prolactin levels and with mRNA expressions of both short and long forms of PRL-R.

CONCLUSION: These results provide evidence, for the first time, that the development of experimental diabetic kidney disease is associated with increases in the renal expression of PRL-R, suggesting a potential role for prolactin in the development and progression of renal injury secondary to diabetes mellitus.

INTRODUCTION

Development of diabetic nephropathy (DN), the leading cause of end-stage renal disease, is characterized by development of albuminuria, glomerulosclerosis, tubulointerstitial degeneration, and fibrosis that is associated with renal dysfunction (Wolf 2004). So far, the complex molecular mechanisms involved in the pathogenesis and progression of DN are not completely known. Therefore, a treatment that can delay the onset of DN and slow its progression is instantly needed to improve the quality of life and survival in patients with diabetes, which is also associated with reduced demands on health resources.

The hormone prolactin, a peptide hormone produced by the anterior pituitary gland, is involved in various actions in the body, including
lactation, water and electrolyte balance, reproduction and immune response (Freeman et al. 2000). Two different prolactin receptor (PRL-R) gene isoforms, short and long, have been identified in the rats' different tissues, including the kidneys (Nagano & Kelly 1994). Although the gene expression of PRL-R long form is predominant compared with short form in many organs, the kidney expressed both forms equally (Nagano & Kelly 1994). In rats' kidney prolactin plays an important role in the regulation of ion transport and renal function. However, there have been conflicting reports on whether prolactin act as antinatriuretic or a natriuretic hormone (Ibarra et al. 2005). The natriuretic effect of prolactin was associated with dose dependently decreased proximal tubule Na-K-ATPase activity (Ibarra et al. 2005).

Disturbances of prolactin secretion occur in both the chronic kidney disease and in diabetes mellitus (Arnold et al. 2010; Rath & Ramachandran 2012; Cowden et al. 1978). In diabetic patients, experimental studies reporting that circulating levels of prolactin are elevated (Arnold et al. 2010; Mooradian et al. 1985). Although no studies to date have addressed the effects of prolactin in DN, there is some evidence to suggest that prolactin may play a role in the DN. Increase in serum prolactin levels in DN is correlates negatively with glomerular filtration rate and positively with creatinine concentrations (Sari et al. 2012). Additionally, Mejia-Rodriguez et al. (2013) have reported that reducing prolactin levels by bromocriptine, a type-2 dopamine receptor agonist, prevented the progression of chronic kidney disease in patients with type 2 diabetes. In the light of the potential clinical relevance of prolactin in DN patients and because the biological effects of prolactin are presumably mediated by the PRL-R, it is relevant to determine whether the altered expression of each receptor subtype occurs in the kidney during the progression of DN. This might be an approach to understand part of the mechanisms involved in the pathogenesis and progression of DN. The present study therefore, was intended to examine whether the renal mRNA expression of PRL-R is altered in experimental diabetes, and how such changes related to the development of albuminuria.

**MATERIALS AND METHODS**

**Induction of diabetes and experimental protocols**

All experimental procedures were pre-approved by the university animal care and use committee. The study was performed in female adult wistar rats, 55–60 days old and weighing approximately 200 g. The rats were housed in a controlled environment at 21–23°C on an illumination schedule of 12 h of light and 12 h of darkness. Standard pellet food and water were provided ad libitum. Diabetes was induced in rats by intera-peritoneal injecting a freshly prepared STZ) Sigma-Aldrich, USA; 55 mg/kg; dissolved in 0.1 M acetate buffer; pH 4.5) after an overnight fast. The rats were randomly divided into two groups (n=6 per group): (1) control (non-diabetic) and (2) diabetic.

**Urine, blood and tissue collection**

After 10 weeks of diabetes, the rats were placed in metabolic cages one day before sacrifice, and urine was collected for 24 h for the analysis of urine albumin concentration and the urine output. Then, the animals were weighed and anesthetized with ketamine and xylazine, and blood samples were collected (via cardiac puncture). The kidneys were removed and transferred into RNAlater solution (Sigma-Aldrich, USA) for the real time PCR analysis. The animals were sacrificed via anesthetic overdose.

**Measurements of blood glucose, urine albumin excretion and serum prolactin level**

Blood glucose level was determined by glucometer (Accu-Chek Performa, Roche Diagnostics). Urine samples were centrifuged at 4°C and 2,000 rpm for 10 min. The urinary albumin concentration in the supernatant was measured using Albumin Rat ELISA kit (Abcam/UK) according to the manufacturer’s protocols and the rate of urine albumin excretion (UAEx) was calculated based on the measured concentrations. Serum prolactin level was measured by ELISA (Cusabio/China), according to the manufacturer’s protocol.

**RNA preparation and reverse transcription**

Total RNA was extracted from RNAlater-preserved kidney tissues using an RNeasy mini tissue kit (Qiagen, USA) according to the manufacture protocols. The resulting RNA pellets were dissolved in RNase-free water and the quantity and quality of the isolated RNA were determined by absorbance at 260 and 280 nm and OD 260/280 nm ratios >1.8 were obtained for all samples, indicating high purity. Samples were then stored at −20°C for subsequent RT-PCR analysis.

Total RNA (0.5 μg) was reversely transcribed using oligo-(dT)15 primer in a 20-μl reaction according to the manufacturer’s instructions (iNTRON Biotechnology/S. Korea). Reverse transcription reactions were carried out at 25°C for 10 min followed by 42°C for 60 min and 95°C for 5 min. The resulting first-strands cDNA were stored at −20°C until use for real time RT-PCR.

**Real time RT-PCR**

Quantitative real time RT-PCR was carried out on LineGene 9600 Real-Time PCR system (Bioro Technology Co, Bingjiang, China), using β-actin as a non-regulated reference gene. Primers were designed and synthesized by IDT (Integrated DNA Technologies, INC). The forward sequence for the primer used for long form of PRL-R (NCBI Genbank Accession No: NM_001034111) was CTCTTCAAGTTGCCCCCTTC and the reverse primer was CATTCTCTCTGCTGTCATCTGT. The forward sequence for the primer used for the short form of PRL-R (NCBI Genbank Accession No:
NM_012630) was CAGATCCACCTTGTATTTGCTTG and the reverse primer was TTGATTATGTCTGGGCAGTG. The forward sequence for β-Actin (NCBI Genbank Accession No: NM_031144) primer was CCTAGACTTCGAGCAAGAGA and the reverse primer was TCCATACCCAGGAAGGA.

The SYBR-PCR reactions were performed using the KAPA SYBR® FAST Universal 2X qPCR master mix (KAPA Biosystem / USA) in 10 μl final volume. The amplification conditions for quantification were: 95 °C for 3 min and 45 cycles of 95 °C for 3s and 60 °C for 20 s. After the amplification efficiency of each target and reference gene was validated, the relative gene expression levels were determined by the ΔΔCT method as described by Livak and Schmittgen (2001). The levels of genes expression were expressed as the normalized ratio of gene expression relative to β-actin mRNA level using one sample from the control group as calibrator.

Statistical analysis
All data will be expressed as means ± SEM. Statistical differences between groups were evaluated by the Student’s t-test. Significance was set at p<0.05. The association between PRL-R mRNA expression and the other variables was evaluated by Pearson’s correlation coefficient. All statistical analysis was performed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS
As shown in Table 1, after 10 weeks of diabetes blood glucose was significantly higher in diabetic than in non-diabetic rats. To evaluate the renal hypertrophy and renal dysfunction, ratio of kidney weight/body weight and UAE were measured, respectively. Diabetic rats showed a significant increase in the kidney weight/body weight ratio (Table 1; p<0.05) as compared with non-diabetic group. A significantly increased level of albuminuria (Table 1; p<0.05) was also evident in the diabetic group compared to the non-diabetic group. There was a significant higher circulating prolactin levels in the in the diabetic group compared to the non-diabetic group (Figure 1; p<0.05).

The mRNA expression of both long and short forms of PRL-R were increased 3.5 folds in the diabetic group in compared to the non-diabetic group (Figure 2 and 3; p<0.05) at the end of the study. UAE was significantly and positively correlated with serum prolactin levels (r=0.54, p<0.05) and with mRNA expressions of both short and long forms of PRL-R (r=0.80, p<0.05; r=0.68, p<0.05, respectively). Moreover, the mRNA expression of both long and short forms of PRL-R were positively correlated with serum prolactin levels (r=0.71, p<0.05; r=0.85, p<0.05, respectively).

| Tab. 1. Metabolic and renal parameters in control and diabetic rats. |
|--------------------|-----------------|
|                    | Non-Diabetic    | Diabetic        |
| Kidney weight/body weight ratio | 0.61±0.02      | 0.97±0.08*      |
| Blood glucose, mmol/l          | 5.3±0.26       | 25.2±2.7*       |
| UAE1, mg/day                    | 1.33±0.26      | 6.60±1.42*      |

Data represent the mean ± SEM.*p<0.05 compared to the non-diabetic group. 1UAE: urine albumin excretion

Fig. 1. Serum prolactin level in non-diabetic and diabetic rats. Data represent the mean ± SEM.*p<0.05 compared to the non-diabetic group.

Fig. 2. Long-form PRL-R mRNA expression normalized to β-actin levels in non-diabetic and diabetic rats. Data represent the mean ± SEM.*p<0.05 compared to the non-diabetic group.

Fig. 3. Short-form PRL-R mRNA expression normalized to β-actin levels in non-diabetic and diabetic rats. Data represent the mean ± SEM.*p<0.05 compared to the non-diabetic group.
DISSCUSSION

In the present study, using real time PCR, we examined for the first time the renal expression of PRL-R gene in diabetes mellitus by comparing the levels of the mRNA expression for the long and short forms of PRL-R in normal and diabetic rats. Diabetic rats used in this study exhibit albuminuria, renal hypertrophy and have elevated serum glucose levels; therefore, we successfully established an experimental animal model of type 1 diabetes possessing DN. A key finding of the present study is that serum prolactin and the expression levels of both short and long forms of renal PRL-R mRNA were significantly increased in the diabetic group in compared to the non-diabetic group. Moreover, the increases in the serum prolactin and the mRNA expression levels of renal PRL-R were significantly and positively correlated with the albuminuria.

Previous results on the association between circulating prolactin levels and diabetes are conflicting. Many studies revealed that prolactin levels of diabetic patients are high (Arnold et al. 2010; Mooradian et al. 1985), but some studies do not support these results (Baxter et al. 1981; Cerasola et al. 1981). Here, we show that the circulating concentration of prolactin was higher in the diabetic in compared to the non-diabetic rats. The cause of the elevated serum prolactin level in diabetics is not clear, however, failure of dopamine to suppress prolactin levels through dopaminergic neuronal activity (Mooradian et al. 1985) and decrease prolactin clearance rate by the kidney (Sari et al. 2012) could be possible explanations for the increased serum prolactin level.

In addition to being synthesized and secreted by lactotrophic cells of the anterior pituitary gland, prolactin gene expression has been confirmed in numerous organs, including the kidneys (Sakai et al. 1999). Similar to prolactin gene expression, the tissue distribution of PRL-R mRNA is extremely broad with various PRL-R isoforms have been confirmed in different organs (Sakai et al. 1999; Bole-Feyosot et al. 1998). In accordance with previous studies by Nagano and Kelly (1994), we found that the mRNA of both PRL-R isoforms, i.e., short and long forms is equally expressed in the rat kidney when compared relative to that of the β-actin housekeeping gene.

The understanding of changes in the renal PRL-R expression is essential to clarify mechanism(s) of actions of hyperprolactinaemia on the diabetic kidney. The mechanisms which mediate the increased expression of PRL-R mRNA in diabetic kidney in the present study remain to be investigated. However, the observed positive correlation between serum prolactin and renal PRL-R mRNA expression supports the concept that the higher level of serum prolactin may contribute, at least in part, to the up-regulation of renal PRL-R mRNA in the diabetic kidney.

In the current study, the positive correlation between UAE and serum prolactin levels and between the UAE and the mRNA expressions of both short and long forms of PRL-R suggests that prolactin influences the progression of DN. Earlier studies showed that exogenous prolactin caused a significant increase in frequency and severity of the chronic progressive nephropathy in the rat (Richardson & Luginbühl 1976). Furthermore, suppression of prolactin secretion using prolactin-lowering dopamine agonist, bromocriptine, protects against ischemia/reperfusion injury in rat kidney (Narkar et al. 2004) and lupus nephropathy in mice (McMurray et al. 1991). Although, no data on prolactin contribution to pathogenesis of DN is available, nephroprotective effect of bromocriptine has been also observed in chronic kidney disease patients with type 2 diabetes (Mejia-Rodriguez et al. 2013). Certainly, the functional significance of induction of both forms mRNA for PRL-R in the diabetic kidney needs to be elucidated further.

In conclusion, the results of this study provide evidence, for the first time, that the development of experimental diabetic kidney disease is associated with increases in the renal expression of PRL-R, suggesting a potential role for prolactin in the development and progression of renal injury secondary to diabetes mellitus.

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Competing interests

The authors declare that they have no competing interests.

REFERENCES

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