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Effect of Long-term Administration of Somatostatin Analogue on Renal Enlargement in Uninephrectomized-diabetic Rats

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Recent study has demonstrated that the long-acting somatostatin analogue administration effectively prevented inital renal growth in diabetic and uninephrectomized rats. In the present study we examined long-term effect of somatostatin analogue (Sandostatin) on renal enlargement in uninephrectomized-diabetic rat5. Animals were divided into 4 groups: (1) normal control rats (C) (n = 7), (2) uninephrectomized rats (NPX) (n = 7), (3) uninephrectomized-diabetic rats (NPX + DM) (n = 7) and (4) NPX + DM rats treated with Sandostatin (NPX + DM + Tx) (n = 9). All animals had free access to diet (50% protein) and water during the experimental period. To the NPX + DM + Tx rats, 2.5 μg of Sandostatin was given subcutaneously twice a day for 8 weeks. Periodic observations were done at 0, 4 and 8 weeks. After 8 weeks. NPX rats (0.540 ± 0.017 (SEM)) had higher fractional kidney weights (FKW) (wet kidney wt/body wt) compared to C rats (0.410 ± 0.014) (p < 0.0005), and both NPX + DM rats (0.983 ± 0.098) and NPX + DM + Tx rats (1.091 ± 0.042) had higher FKW compared to C rats (p < 0.0001) and NPX rats (p < 0.005), respectively. But no significant change of FKW was observed between NPX + DM rats and NPX + DM + Tx rats. Systolic blood pressure, BUN, serum creatinine, glomerular filtration rate and 24 hour urine protein excretion in NPX + DM rats were not different from those in NPX + DM + Tx rats. Light microscopically the index of mesangial expansion (IME) was determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus. In the each 50 biopsies, the IME in both NPX + DM rats (0.957 ± 0.147) and NPX + DM + Tx rats (1.366 ± 0.104) were higher than those in NPX rats (0.183 ± 0.030) (p < 0.01) and C rats (0.156 ± 0.028) (p < 0.01), respectively, with no difference between NPX + DM rats and NPX + DM + Tx rats. The size of renal corpuscles of NPX + DM rats was also not different from that of NPX + DM + Tx rats. These results suggest that long-term administration of a somatostatin analogue (5μg of Sandostation per day) can not prevent renal enlargement in uninephrectomized-diabetic rats.

Key Words: *somatostatin, uninephrectomized-diabetic rat, fractional kidney weight, proteinuria, mesangial expansion, size of renal corpuscle*

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INTRODUCTION

Diabetic nephropathy, a major cause of death in diabetes, is the second leading primary renal disease of dialysis patients in Korea, and its proportion in dialysis patients is increasing annually (Kim & Bang, 1991). Diabetic nephropathy develops in 25 to 50 percent of patients with insulin-dependent diabetes (IDDM) (Krolewski et al., 1987). In such patients clinically detectable proteinuria develops 15 to 25 years after onset of diabetes (Krolewski et al., 1987) and azotemia occurs 4 to 5 years later (Hasslacher et al., 1985). This complication of diabetes mellitus, once manifests, is irreversible and its progression cannot be arrested, although it may be retarded with antihypertensive treatment and strict glycemic control (Parving et al., 1983; Viberti et al., 1982; Wiseman et al., 1985).

Generalized renal growth is a characteristic of early diabetes. The stimuli to renal growth in diabetes are not precisely identified, although some of the factors involved in the hemodynamic changes probably contribute. Elevated glomerular filtration rate has been proposed as having a role in the initiation and evolution of diabetic nephropathy (Mogensen & christensen, 1984), and this elevated glomerular filtration rate seems to be correlated closely with increased kidney volume (Wiseman & Viberti, 1983). So, it is very important to find therapeutic strategies to reduce glomerular hyperfiltration and accompanying renal growth in diabetes. Intravenous somatostatin resulted in falls in both glomerular filtration rate (GFR) and renal perfusion without altering systemic blood pressure and cardiac output in anesthetized dogs (Price et al., 1983), and decreased creatinine clearance in man (Walker et al., 1985). Also somatostatin and its analogue resulted in a reduction in both GFR and renal plasma flow in normal subjects and acutely reduced glomerular hyperfiltration in patients with IDDM (Vora et al., 1987). So somatostatin analogue may directly decrease GFR and thus lower urinary protein excretion and prevent renal growth in diabetes.

Recently the role of growth factors in mediating renal growth has been extensively studied. Flyvbjerg et al., (1988, 1989) demonstrated that the initial kidney growth seen in diabetic and uninephrectomized rats is preceded by an increase in kidney tissue somatomedin C content and somatostatin analogue prevents increase in kidney somatomedin C content and initial renal growth in diabetic and uninephrectomized rats suggesting that the growth factor may be responsible for renal growth.

We investigated the effects of long-term administration of somatostatin analogue on the renal growth and the course of nephropathy in uninephrectomized-diabetic rats.

MATERIALS AND METHODS

Male Wistar rats (body weight: 200-250g) were studied. Baseline systolic blood pressure (SBP) by tail cuff method, blood urea nitrogen (BUN), serum creatinine (SCr) and 24 hr urinary protein excretion were obtained. After baseline studies, right kidney was removed through a right flank incision under anesthesia with either and weighed after being trimmed of fat, hilus and capsule. Diabetes was induced 7 days after uninephrectomy by intravenous injection of streptozotocin at a dose of 50mg/kg body weight. All animals had free access to 50% protein diet and water throughout the entire experimental period.

Protocol

The following four groups were studied: I) normal control rats (C, n=7); II) uninephrectomized rats (NPX, n=7); III) uninephrectomized-diabetic rats (NPX+DM, n=7); IV) uninephrectomized-diabetic rats treated with Sandostatin (NPX+DM+Tx, n=9). In diabetic rats, Blood glucose was measured weekly in tail blood (Glucometer II model 5550, Ames division, USA) and maintained between 200 and 400 mg/dl by subcutaneous injection of NPH insulin (Novo, Denmark). Sandostain (Sandoz, Ltd., Basel, Switzerland) was used as a long acting somatostatin analogue and was given subcutaneously to the NPX+DM+Tx rats at a dose of 2.5μg twice a day (9:00 and 16:00) for 8 weeks. The Sandostatin treatment was started immediately after uninephrectomy and the day when the streptozotocin was injected to the rats was defined as the starting day of the study. The dose of Sandostatin used in this study was determined largely based on that decreasing creatinine clearance and GFR in man (Walker et al, 1985; Vora et al., 1987). Blood sample from the retrobulbar venous plexus of lightly anesthetized rats to measure BUN and SCr, and 24 hr urinary collections to measure protein excretion in metabolic cages were performed periodically (week 0, 4 and 8). At the same time, SBP was measured by tail cuff method. At the end of week 8, glomerular filtration rate (GFR) was measured and then left kidney was removed for histological analysis.

Laboratory Methods

Bun was determined by urease-indophenol method using commercially available kits (Eiken, Ltd., Tokyo, Japan). SCr was measured by the method of Jaffe us-

ing commercially available kits (Eiken, Ltd., Toky, Japan). Urinary protein was determined by turbid method using 3% sulfosalycilic acid. To measure GFR, catheters were placed in the jugular vein, carotid artery and bladder. 100μCi of ^{99m}Tc-DTPA was mixed in 10ml of normal saline and infused into the jugular vein at the rate of 2.2ml/hr with a SAGE automatic infusion pump. After a 45 minutes stabilization period, GFR was determined every 10 min for 30 min (Lee & Blaufox, 1987).

Kidney weight, fractional kidney weight (FKW) and compensatory renal growth (CRG)

Removed left kidney was trimmed of fat, hilus and capsule and then weighed. FKW was calculated as percentage of kidney weight to the body weight in each rat. CRG was assessed by comparing the right wet kidney weight (RKW) at nephrectomy to left wet kidney weight (LKW) at sacrifice in each rat. CRG was expressed in percentage terms as the difference in weight between the left and right kidney, as a proportion of the weight of the right kidney, according to the formula:

$$CRG = \frac{LKW\text{-}RKW}{RKW} \times 100$$

In order to allow for any loss in body weight during the experimental period, a further comparison was made between right FKW at nephrectomy and left FKW at sacrifice in each nephrectomized rat, using the same formula.

Histological Analyses

The left kidney was immersion fixed in 10% formaline. Sections were cut at 3 μm and stained with periodic acid-Schiff. Index of mesangial expansion was determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus (Mauer et al., 1984). O was used as normal, 1.0 as twice the normal thickness, 2.0 as three times the normal thick- etc. Half grades were assigned where appropriate (Fig. 1A, B, C). More than 50 glomeruli were examined in each rat. Size (cross-sectional area) of renal corpuscles was computed by using an image analysis system (Vidas 2.0, Kontron Bildanalyse GMBH, Germany). More than 100 renal corpuscles were computed in each group.

Statistical analysis

The data are expressed as mean ± SEM unless stated otherwise. Differences between the groups were analysed by repeated measures ANOVA in combination with the Scheffe's test and differences in each group according to the time interval were analysed by Student's t-test in combination with Bonferroni test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

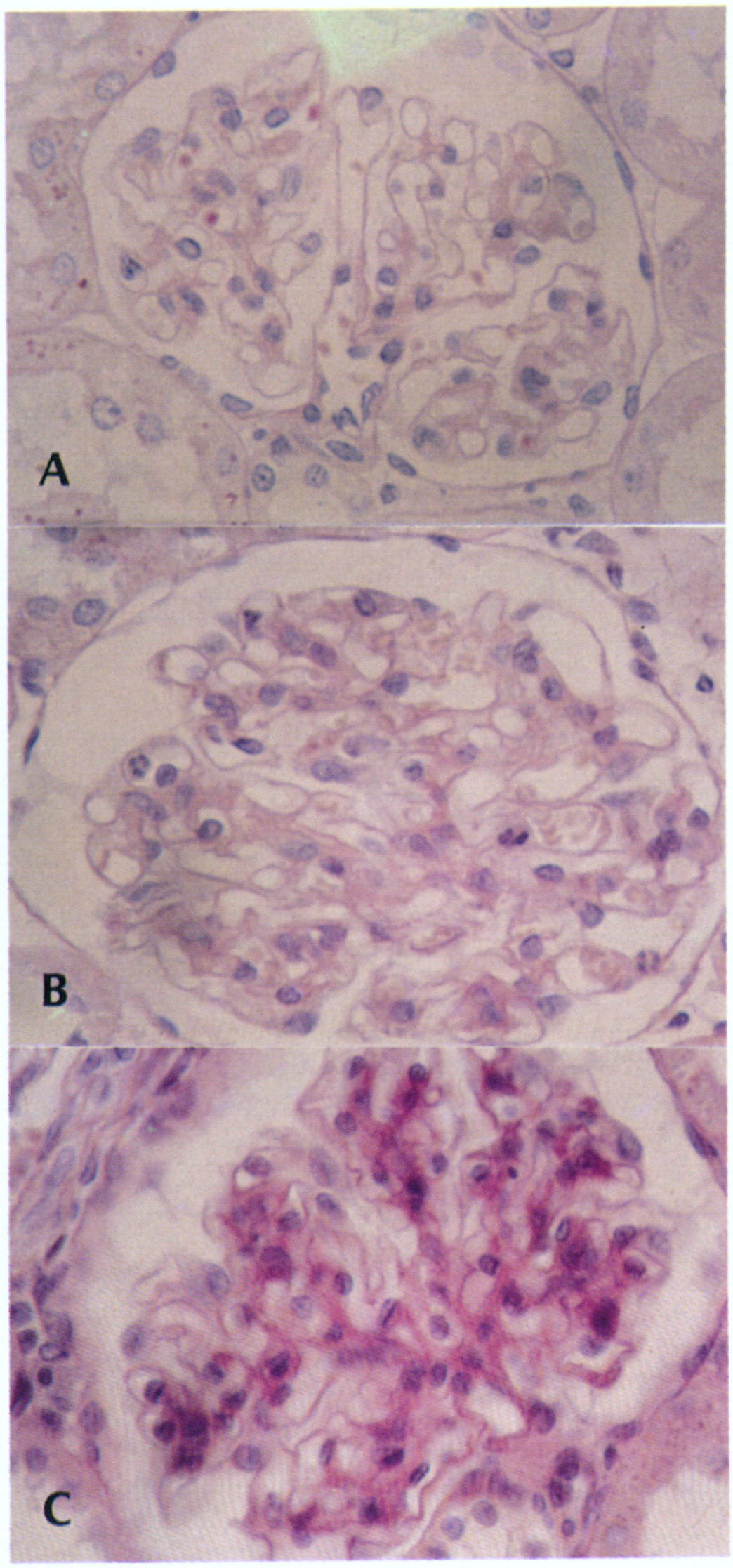

Fig. 1. Microphotograph of glomerulus of rat. (A) Normal glomerulus. Index of mesangial expansion scored O. (B) Glomerulus whose mesangium is mildly expanded. Index of mesangial expansion scored 1.0. (C) Glomerulus whose mesangium is severely expanded. Index of mesangial expansion scored 2.0 (PAS stain, x 400)

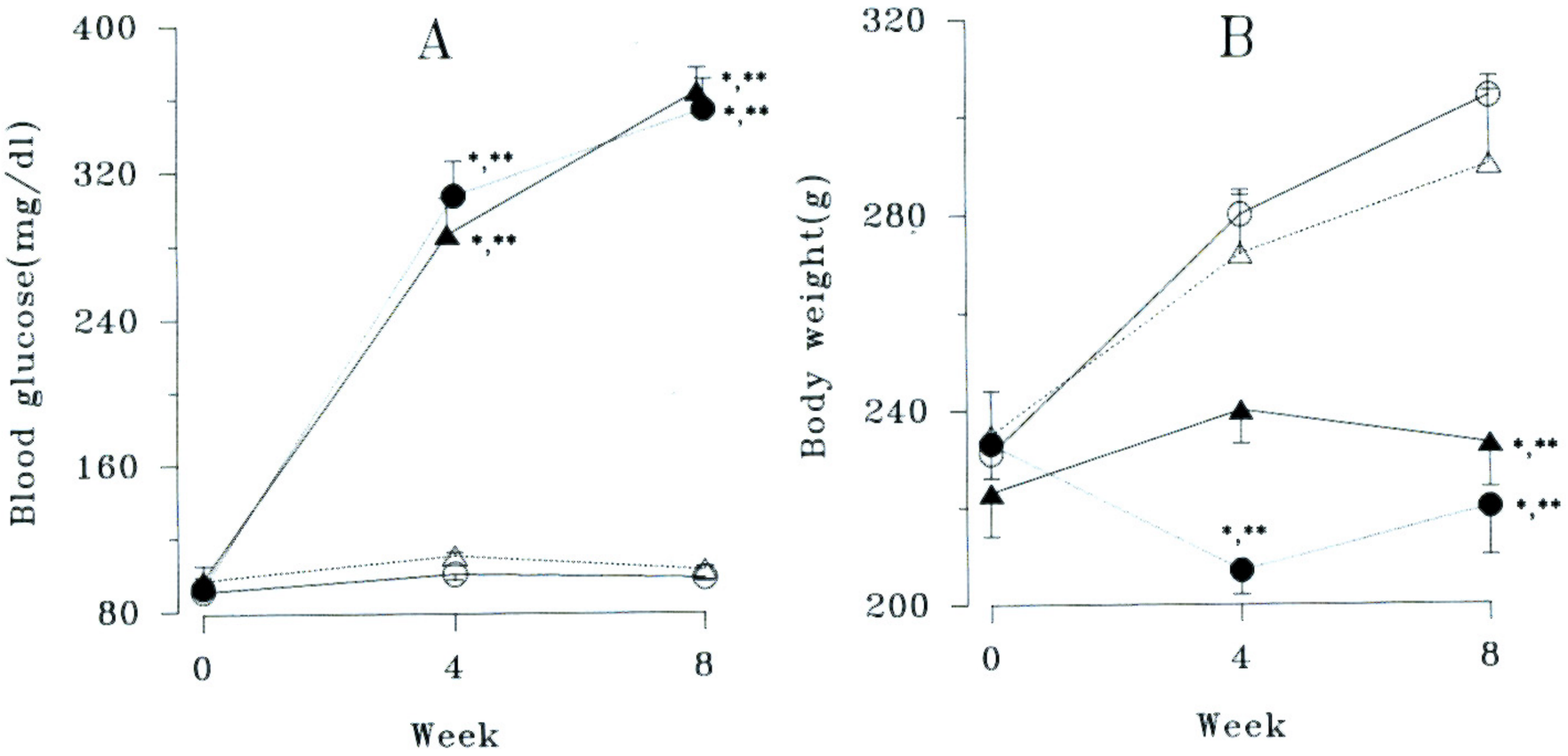

Fig. 2. Changes in blood glucose (A) and body weight (B) during the study. ○ Normal control rat, △ Uninephrec tomized rat, ▲ Uninephrectomized-diabetic rat, ● Uninephrectomized-diabetic rat treated with Sandostatin *: $p<0.01$ vs. normal control rat, **: $p<0.01$ vs. uninephrectomized rat

Table 1. Systolic blood presure and renal function during the study.

	C (n=7)	NPX (n=7)	NPX+DM (n=7)	NPX+DM+Tx (n=9)
SBP (mmHg)				
Week 0	127±3	121±3	126±3	120±4
4	129±5	132±5	130±5	128±3
8	130±4	145±4[A]	154±2 [A]	137±4
p*	NS	<0.001	<0.001	<0.05
BUN (mg/dl)				
Week 0	22.9±0.6	23.6±1.6	22.6±1.5	22.1±0.9
4	29.9±0.6	36.1±1.5	41.1±1.9	47.5±3.1[A]
8	30.7±0.6	42.7±1.5[A]	43.7±1.8	51.7±1.3[A]
p*	<0.0001	<0.0001	<0.0001	<0.0001
SCr (mg/dl)				
Week 0	0.73±0.03	0.69±0.01	0.72±0.02	0.75±0.01
4	0.80±0.01	0.78±0.03	1.00±0.01 [A]	1.04±0.04[A, B]
8	0.77±0.01	0.87±0.02[A]	1.05±0.01[A, B]	1.04±0.03[A]
p*	NS	<0.0001	<0.0001	<0.0001
GFR (ml/min)				
Week 8	1.42±0.23	1.29±0.03	0.98±0.20[A, B]	0.94±0.07[A, B]

Abbreviations: SBP, systolic blood pressure: SCr, serum creatinine: GFR, glomerular filtration rate: C, normal control rat: NPX, uninephrectomized rat: NPX+DM, uninephrectomized-diabetic rat: NPX+DM+Tx, NPX+DM rat treated with Sandostatin . (p*:p value (week 0 vs. week 8), [A]: $p<0.05$ vs. C, [B]: $p<0.05$ vs. NPX)

RESULTS

Blood glucose and body weight (Fig. 2)

NPX+DM and NPX+DM+Tx rats developed diabetes one day after induction of diabetes and insulin requirement was 1-2 U (NPH insulin) in each day to maintain the blood glucose values between 200 and 400 mg/dl (Fig. 2A). Those rats had significantly low-

er body weights compared with C and NPX rats during the experimental period ($p<0.05$) (Fig. 2B). NPX+DM+Tx rats had a weight loss of 11% at week 4 from initial level followed by a weight gain back toward initial level at the end of the study.

Blood pressure and renal function (Table 1)

SBP in C rats remained unchanged throughout the entire experimental period. On the other hand, SBP in NPX ($p<0.001$), NPX+DM ($p<0.001$) and NPX+DM+Tx ($p<0.05$) rats elevated at week 8 compared with week 0, respectively. SBP in NPX ($p<0.05$) and NPX+DM ($p<0.05$) rats became significant higher than those in C rats at week 8, respectively.

Bun elevated significantly in all rats at week 8 compared with week 0 ($p<0.0001$), and became significantly higher in NPX+DM+Tx rats ($p<0.05$) at week 4 and in NPX ($p<0.05$) and NPX+DM+Tx ($p<0.05$) rats at week 8 than that in C rats. SCr also elevated significantly in all rats except C rats at week 8 compared with week 0 ($p<0.0001$), and became significantly higher in NPX+DM and NPX+DM+Tx rats at week 4 and in NPX, NPX+DM and NPX+DM+Tx at weeks 8 than that in C rats ($p<0.05$, respectively).

GFR measured at week 8 in both NPX+DM and NPX+DM+Tx rats were significantly lower than those in C ($p<0.05$) and NPX ($p<0.05$) rats. There were no difference in SCr (1.05 ± 0.01 vs. 1.04 ± 0.03 mg/dl at week 8) and GFR (0.98 ± 0.20 vs. 0.94 ± 0.07ml/min) between NPX+DM and NPX+DM+Tx rats.

Urinary protein excretion (Table 2)

24 hr urinary protein excretion increased significantly in all rats at week 8 compared with week 0 ($p<0.05$). The NPX+DM rats tended to excrete urinary protein more than C and NPX rats did, though not significant statistically. There was no difference in urinary protein excretion between NPX+DM and NPX+DM+Tx rats.

Kidney weight, FKW and CRG (Table 3)

FKW of right kidneys at nephrectomy were similar in NPX, NPX+DM and NPX+DM+Tx rats. On the other hand, left FKW at sacrifice of NPX rats (0.540 ± 0.017) was higher than that of C rats (0.410 ± 0.014) ($p<0.01$) and those of both NPX+DM and NPX+DM+Tx rats (0.983 ± 0.098 and 1.091 ± 0.042, respectively) were even higher than those of C ($p<0.001$) and NPX ($p<0.001$) rats. Also, CRG was significantly higher in both NPX+DM and NPX+DM+Tx rats than in NPX rats ($p<0.001$, respectively). But CRG of NPX+DM rats did not differ from that of NPX+DM+Tx rats.

Histological analyses (Table 4)

Indices of mesangial expansion in both NPX+DM

Table 2. Urinary protein excretion during the study (mg/24h)

	C (n=7)	NPX (n=7)	NPX+DM (n=7)	NPX+DM+Tx (n=9)
Week 0	16.6±2.3	22.3±4.1	19.0±3.3	20.7±1.3
4	28.5±3.2	25.8±3.9	28.5±5.9	31.0±4.9
8	40.1±2.8	39.6±2.9	63.0±7.0	50.1±9.1
p*	<0.0001	<0.05	<0.0001	<0.05

Abbreviations are same as table 1. p*: p value (week 0 vs. week 8)

Table 3. Kidney weight, fractional kidney weight (FKW) and compensatory renal growth (CRG)

	RKW (g)	FKW 1	LKW (g)	FKW 2	CRG1 (%)	CRG 2 (%)
C (n=7)			1.124±0.028	0.410±0.014		
NPX (n=7)	0.781±0.026	0.322±0.013	1.514±0.064	0.540 ± 0.017^{A}	92.64±6.33	70.78±13.40
NPX+DM (n=7)	0.737±0.021	0.325±0.012	2.242±0.148	$0.983\pm0.098^{A,B}$	204.02 ± 17.99^{B}	200.72 ± 25.32^{B}
NPX±DM+Tx (n=9)	0.799±0.022	0.323±0.004	2.374±0.069	$1.091\pm0.042^{A,B}$	198.98 ± 12.01^{B}	238.22 ± 1.43^{B}

Values are expressed as mean±SEM. ^{A}: $p<0.01$ vs. C, ^{B}: $p<0.01$ vs. NPX

RKW: Right kidney weight at nephrectomy, LKW: Left kidney weight at sacrifice

FKWI: RKW/100g, body weight, FKW2: LKW/100g, body weight

CRGI: (LKW-RKW)/RKW×100. CRG2: (FKW2-FKW1)/FKW1×100

Table 4. Histological analyses

	C	NPX	NPX+DM	NPX+DM+Tx
Index of mesangial expansion	0.16±0.07	0.18±0.08	0.96±0.39[A,B]	1.37±0.31[A,B]
Size of renal corpuscles ($\times 10^2$ μm^2)	72.5±18.2	79.7±17.7	85.0±23.2[A]	81.2±22.0[A]

Abbreviations are same as table 1. Values are expressed as mean±SD
[A]: $p<0.05$ vs. C, [B]: $p<0.05$ vs. NPX.

(0.96±0.39) and NPX+DM+Tx (1.37±0.31) rats were significantly higher than those in C (0.16±0.07) and NPX (0.18±0.08) rats ($p<0.01$, respectively). Sizes of renal corpuscles in both NPX+DM (8500±2320μm^2) and NPX+DM+Tx (8120±2200μm^2) rats were also significantly larger than those in C (7250±1820μm^2) rats ($p<0.01$, respectively). There was no difference in index of mesangial expansion or size of renal corpuscles between NPX+DM and NPX+DM+Tx rats.

DISCUSSION

The present study demonstrates that diabetes of recent onset as well as unilateral nephrectomy are followed by renal growth and that uninephrectomy has additive effects to diabetes on the deterioration of renal function and renal growth. Another finding is that chronic administration of somatostatin analogue, Sandostatin, at the dose of 5μg/day does not prevent the deterioration of renal function and renal growth in uninephrectomized-diabetic rats during the 8 weeks' observation. In this study, we used high protein diet in order to enhance hyperfiltration and to observe the course of overt diabetic nephropathy.

It has been suggested that glomerular hyperfiltration includng increased single nephron GFR seen early in the course of diabetes, whatever its cause, plays an important role in late diabetic nephropathy (Brenner et al., 1981; Mogensen, 1986). The preclinical stage of diabetes, characterized by elevation of GFR, increase in renal plasma flow, microalbuminuria and increase in kidney size is followed by the clinica stage. haralded by the development of persistent, easily measurable proteinuria and declining GFR. In this study, BUN and serum creatinine levels were elevated and GFR was declined in NPX+DM and NPX+DM+Tx rats at week 8, which illustrated that those rats were in the clinical stage of diabetic nephropathy. And the higher serum creatinine levels of NPX+DM and NPX+DM+Tx rats compared with NPX rats were considered to present an additive effect of uninephrectomy to diabetes on the renal functional deterioration. Therefore unilateral nephrectomy is considered to be a risk factor for the progression of nephropathy in diabetic patients. High protein diet used in this study might play a role in the progression of renal functional deterioration through enhancing hyperfiltration because normal control rats also showed high BUN levels at weeks 8. Several factors have been investigated as possible mediators of glomerular hyperfiltration in diabetes. These include hyperglycemia, increased glomerular prostaglandin production, decreased sensitivity of the transforming growth factor mechanism, elevated levels of atrial natriuretic peptide, altered glomerular myoinositol metabolism, and increased glomerular filtration surface area (O'Donnell et al., 1988). It has been demonstrated that somatostatin infusion results in a marked and rapid decrease in renal plasma flow (RPF) and, as a consequence, GFR (Vora et al., 1987), and that somatostatin decreases urinary flow, and increases urinary osmolality due to a marked and prompt reduction of RPF and GFR without alteration of plasma arginine vasopressin levels (Walker et al., 1985). In our study, lower GFR in both NPX+DM and NPX+DM+Tx rats than in C rats was considered to reflect higher serum creatinine and no difference in GFR was found whether Sandostatin was given or not.

Moderate arterial hypertension is present in many young insulin dependent diabetic patients (Parving et al., 1983). Hasslacher et al. (1985) demonstrated that pre-proteinuric type I diabetic patients who subsequently developed clinical nephropathy had a slight, but significant, elevation of systolic blood pressure and a higher prevalence of established hypertension compared with long-standing type I diabetic patients without subsequent nephropathy. And arterial blood pressure seems to have a complex relation with diabetic nephropathy-nephropathy raising blood pressure and blood pressure accelerating the course of

nephropathy, and early aggressive antihypertensive treatment reduces the rate of decline in kidney function in diabetic nephropathy (Parving et al., 1983). In the present study, elevation of systolic blood pressure in NPX, NPX+DM and NPX+DM+Tx rats during the study might result initially from the nephropathy, and then might accelerate nephropathy.

Increased uninary albumin excretion is a strong predictor of the development of overt nephropathy in insulin dependent diabetic patients (Mogensen & Christensen, 1984). Viberti et al., (1982) have reported that patients with longstanding IDDM and urinary albumin excretion rates >30μg/min (or >42 mg/24hr) have a higher risk of progressive renal dysfunction. On the other hand, Mauer et al. (1984) have demonstrated that urinary albumin excretion rates >42 mg/24hr but <400 mg/24hr were not necessarily associated with more advanced glomerular changes in their study about the structural-functional relationships in diabetic nephropathy. In our study, 24hr urinary protein excretion increased during the study in all rats including normal control rats, which might be due to massive protein loading from the diet. But it was unclear why the rate of increase in protein excretion was not different significantly between NPX+DM and C rats, although mesangial expansion or size of renal corpuscles were different significantly between those rats. The mean of urinary protein excretion at week 8 ranged between about 40 and 60mg/24hr and this discrepancy between proteinuria and histological findings was the same as the result of the study by Mauer et al. (1984). And significant difference in protein excretion between NPX+DM and C rats might be found if the observation period was longer, because increasing rate of protein excretion in NPX+DM rats was higher than that in C rats. Mogensen and Christensen (1984) have suggested the criteria of a urinary albumin excretion rate at or above 15μg per minute, a glomerular filtration rate above 150ml per minute, and a diastolic pressure at or above 90mmHg as a "predictive index" for the progression to overt nephropathy.

In streptozotocin or alloxan-induced diabetic rats, the weight of the kidney increases within 60 hours of the appearance of hyperglycemia or glucosuria, and the kidney weight increases by 15% to 20% 3 days after administration of streptozotocin and by 70% to 90% after 6 weeks of diabetes (Seyer-Hansen, 1983). The increase in kidney weight is due to cellular hypertrophy and hyperplasia. The stimuli to renal growth in diabetes are not precisely identified. But the hormones such as growth hormone and glucagon as well as hemodynamic change have been considered to contribute to renal growth. Recently it has been suggested that local production of somatomedin C might be involved in initial diabetic and post-nephrectomy growth (Flyvbjerg et al., 1988; Flyvbjerg et al., 1989). Somatomedin C is a polypeptide with potent migogenic activity in vitro (Clemmons et al., 1981) and can be synthesized in the kidney (Flyvbjerg et al., 1990.)

Somatostain secretory cells have been identified in the rat glomerulus (Kurokawa et al., 1983). The effects of somatostatin and its synthetic analogues on kidney function are suppression of hyperfiltration and nenal plasma flow and reduction in initial renal hypertrophy in diabetic patients (Vora et al., 1987), decreasing fasting blood glucose, reduction in postprandial hyperglycemi a in diabetic patients, inhibition of growth hormone secretion and suppression of increase in kidney somatomedin C in diabetes (Flyvbjerg et al., 1989). Bearing in mind the above mentioned wide suppressive action of somatostatin, we investigated the long-term effect of somatostatin analogue on the renal growth in uninephrectomized-diabetic rats. In contrast to other studies (Flyvbjerg et al., 1989; uemasu et al., 1990: Vora et al., 1987), Sandostatin treatment for 8 weeks did not prevent renal growth in uninephrectomized-diabetic rats. Several factors might be possible causes of this different result. First, renal growth is related to complex factors as the nephropathy progresses. The effects of partially suppressed hemodynamic and hormonal factors might not be reflected in the study by Flyvbjerg et al. (1989) because the study was performed for only 4 days. Secondly, the dosage of Sandostatin applied in our study might be too small. The dosage applied in the study by flyvbjerg et al. (1989) was 200μg daily, which was a much higher dosage than ours by 40 times. Finally, the high protein diet might have masked the suppressive effect of Sandostatin on hyperfiltration in our study. To gain further insight, we are currently investigating the same model on a 20% protein diet and a higher dosage of Sandostatin.

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