Renal histology and morphometric analysis Kidney sections were fixed by immersion in Carnoy's solution, followed by 4% buffered formaldehyde, and embedded in paraffin. Sections (2 μm thick) were stained with periodic acid–Schiff and examined by light microscopy. Measurement of the glomerular cross-sectional area and the mesangial area of 30 glomeruli randomly selected in each mouse by scanning of the outer cortex was performed with a computer-aided manipulator (KS-400; Carl Zeiss Vision, Munich, Germany) [23, 24].

Immunohistochemistry For immunohistochemical study of TGF-β, the kidney sections embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetechnical, Tokyo, Japan) were snap frozen in acetone/dry ice, and 4-μm-thick cryostat sections were fixed in acetone [23]. The sections were washed with phosphate-buffered saline, and treated with 0.9% H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity. The specimens were incubated overnight at 4°C with rabbit anti-mouse TGF-β antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After incubation with biotin-conjugated second antibody, the specimens were processed by use of an avidin–biotin–peroxidase complex kit (Vector, Burlingame, CA, USA) and developed with 3,3′-diaminobenzidine tetrahydrochloride (Kanto Chemical, Tokyo, Japan).

Cell culture Mesangial cells were established from glomeruli isolated from 10-week-old male Sprague–Dawley rats using a differential sieving method [25, 26] and used at passages 7–10. Mesangial cells were identified by immunofluorescence techniques [26]. Cells were grown in DMEM (Gibco BRL, Grand Island, NY, USA) containing 20% FCS (Sanko Junyaku, Tokyo, Japan). As the cells reached 80% confluence, they were grown in DMEM containing 10% FCS supplemented with 5.6 mmol/l glucose (normal glucose), 25 mmol/l glucose (high glucose), or 5.6 mmol/l glucose plus 19.4 mmol/l mannitol (as an osmotic control) for 5 days. After this time, the medium was changed to DMEM containing 0.2% FCS supplemented with 5.6 mmol/l glucose, 25 mmol/l glucose, or 5.6 mmol/l glucose plus 19.4 mmol/l mannitol for 24 h. Then, in the presence or absence of rat BNP (100 nmol/l) (Peptide Institute, Osaka, Japan) or 8-bromo-cGMP (1 mmol/l) (Sigma), the cells were further incubated for 24 h. Phorbol 12-myristate 13-acetate (100 nmol/l) (PMA; Sigma) was used for the activation of PKC [26].

Northern blot analysis Total RNA was extracted from whole kidney and mesangial cells by the acid guanidinium–phenol–chloroform method and used for northern blot analysis as described previously [24, 25]. In brief, 25 μg of total RNA was electrophoresed on 1.1% agarose gels containing 2.2 mol/l formaldehyde, and RNA was transferred onto nylon membrane filters. The cDNA fragments corresponding to genes for rat TGF-β_1 (*Tgfb1*, nt 1142–1546), rat fibronectin (*Fn1*, nt 619–1082), mouse α1(IV) collagen (*Col4a1*, nt 5808–6165) and mouse TGF-β_1 (*Tgfb1*, nt 1141–1549), which were prepared by RT-PCR using rat and mouse kidney mRNA, were used as probes. The filter was hybridised with radiolabelled probes and autoradiography was performed using a BAS-2500 bio-imaging analyser (Fuji Photo film, Tokyo, Japan). The filters were rehybridised with human *GAPDH* cDNA probe for normalisation.

Western blot analysis Whole-kidney tissues and mesangial cells were lysed on ice in lysis buffer containing 1 mol/l Tris–HCl (pH 7.5), 12 mmol/l β-glycerophosphate, 0.1 mol/l EGTA, 1 mmol/l pyrophosphate, 5 mmol/l NaF, 10 mg/ml aprotinin, 2 mmol/l dithiothreitol, 1 mmol/l sodium orthovanadate, 1 mmol/l phenylmethylsulfonyl fluoride, and 1% Triton X-100 [24]. The lysates were centrifuged at 15,000 ×g for 20 min at 4°C, and supernatants mixed with Laemmli's sample buffer (40 μg protein/lane) were separated by 12.5% SDS-PAGE

Table 1 Characteristics of control and diabetic mice at 16 weeks

	Control		Diabetes	
	non-Tg (n=5)	BNP-Tg (n=8)	non-Tg (n=10)	BNP-Tg (n=10)
Blood glucose (mmol/l)	8.5±1.7	9.8±1.0	27.2±1.8^b	31.1±0.9^c
Systolic blood pressure (mmHg)	112±3	90±2^b	108±2	92±2^d
Body weight (g)	31.9±1.2	34.4±1.5	27.4±1.0^b	31.6±0.5^c
Kidney weight (g)	0.20±0.01	0.21±0.01	0.22±0.01	0.20±0.01
Kidney/body weight (%)	6.30±0.46	5.93±0.30	7.96±0.47^a	6.37±0.28^d

Values are expressed as means±SEM
$^a p<0.05$ vs control non-Tg mice
$^b p<0.005$ vs control non-Tg mice
$^c p<0.005$ vs control BNP-Tg mice
$^d p<0.05$ vs. diabetic non-Tg mice

and electrophoretically transferred onto Immobilon filters. The filters were incubated with antibodies against total extracellular signal-regulated kinase (ERK)-1/2 or phosphorylated ERK-1/2 (New England Biolabs, Boston, MA, USA) for 2 h at room temperature, and immunoblots were developed with horseradish peroxidase-conjugated donkey anti-rabbit IgG (Bio-Rad, Richmond, CA, USA) and a chemiluminescence kit (ECL One Plus; Amersham, Arlington Heights, IL, USA).

Statistical analysis Data are expressed as means±SEM. Statistical analysis was performed by ANOVA followed by Scheffe's test. A p value of <0.05 was considered statistically significant.

Results

Characteristics of diabetic mice At baseline, there was no significant difference between BNP-Tg and non-Tg mice in terms of blood glucose levels (9.1±0.4 vs 8.5±1.1 mmol/l, n=10 per group), body weight or kidney weight (data not shown). After induction of diabetes, both BNP-Tg and non-Tg mice exhibited sustained hyperglycaemia, with no significant difference in blood glucose levels between them (Table 1). At baseline, the blood pressure of the BNP-Tg mice was 15–20 mmHg lower than that of the non-Tg mice, as observed previously [23, 24], and remained significantly lower during the study (Table 1). There were no significant blood pressure changes in the two groups following the induction of diabetes. At 16 weeks, diabetic non-Tg mice showed renal hypertrophy, as indicated by an increase in kidney weight per body weight, whereas BNP-Tg mice did not (Table 1).

Renal function and proteinuria of diabetic mice To evaluate the functional alterations in the kidneys of diabetic mice, we examined urinary albumin excretion and serum creatinine and urea nitrogen levels, together with calculated creatinine clearance. At baseline, there were no significant differences in these parameters between control non-Tg and control BNP-Tg mice (Fig. 1a; see [23]). After induction of diabetes, urinary albumin excretion of diabetic non-Tg mice markedly increased by 4 weeks, peaked at 12 weeks, and remained significantly elevated until 16 weeks (23.4±6.4, 142.7±33.1, 264.3±46.5, 290.3±55.0, and 181.5±22.7 µg/day at 0, 4, 8, 12 and 16 weeks, respectively, n=10). In diabetic BNP-Tg mice, on the other hand, the increase in urinary albumin excretion was much attenuated and significantly milder than that observed in diabetic non-Tg mice at 8, 12 and 16 weeks (31.1±6.6, 100.8±22.6, 136.6±24.5, 144.1±18.6, and 120.6±19.2 µg/day at 0, 4, 8, 12 and 16 weeks, respectively, n=10) (Fig. 1a). Urinary albumin excretion adjusted for creatinine was also significantly lower (~50%) in diabetic BNP-Tg mice than in diabetic non-Tg mice and was not significantly different from that seen in control non-Tg mice (Fig. 1b).

After 16 weeks of diabetes, non-Tg mice exhibited significantly increased serum creatinine and urea nitrogen levels, together with reduced creatinine clearance (Table 2). In contrast, these parameters were not significantly different from controls in diabetic BNP-Tg mice (Table 2). Thus, functional impairment became manifest at the chronic

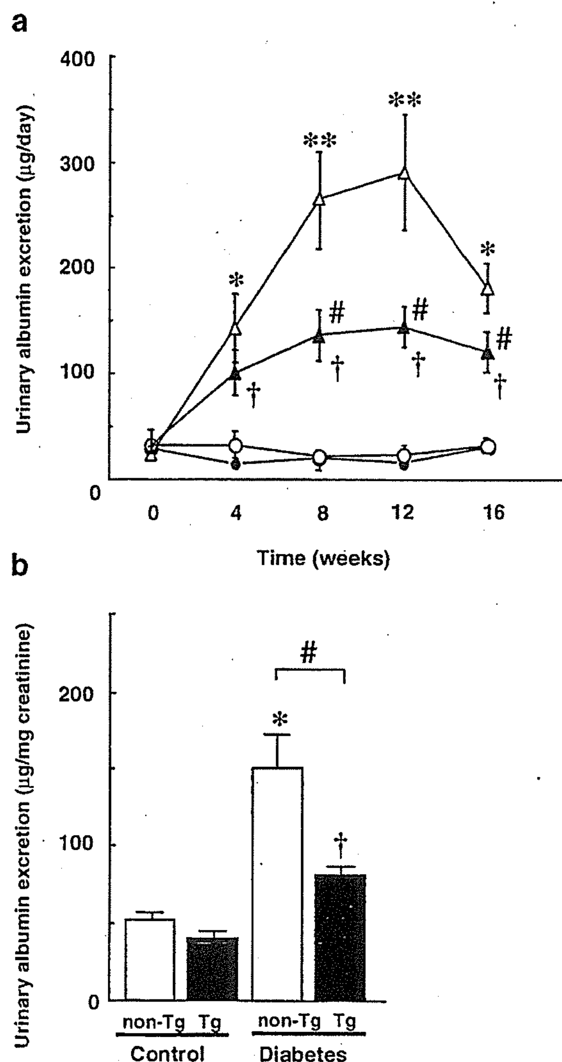


Fig. 1 **a** Daily urinary albumin excretion of control and diabetic mice at 0, 4, 8, 12 and 16 weeks after induction of diabetes. *Open circles*, control non-Tg mice (n=5); *closed circles*, control BNP-Tg mice (n=8); *open triangles*, diabetic non-Tg mice (n=10); *closed triangles*, diabetic BNP-Tg mice (n=10). **b** Urinary albumin excretion normalised to creatinine at 16 weeks. *p<0.05, **p<0.02 vs control non-Tg mice; †p<0.05 vs control BNP-Tg mice; #p< 0.05 vs diabetic non-Tg mice

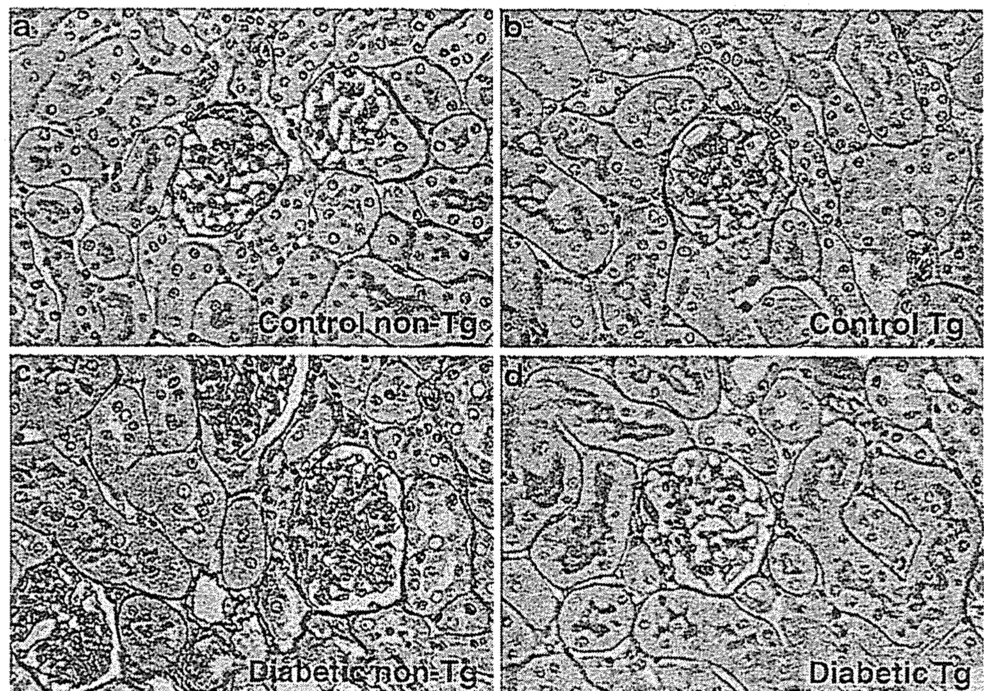
Table 2 Renal function of control and diabetic mice at 16 weeks

Parameter	Control		Diabetes	
	non-Tg (*n*=5)	BNP-Tg (*n*=8)	non-Tg (*n*=10)	BNP-Tg (*n*=10)
Serum creatinine (μmol/l)	8.8±0.9	9.7±1.8	18.6±1.8[b]	12.4±0.9[c]
Blood urea nitrogen (mmol/l)	11.4±0.9	11.2±0.9	16.8±1.4[a]	14.6±1.6
Creatinine clearance (ml/min)	0.56±0.11	0.57±0.10	0.30±0.05[a]	0.54±0.06[c]

Values are expressed as means±SEM
[a]$p<.0.05$ vs control non-Tg mice
[b]$p<0.02$ vs control non-Tg mice
[c]$p<0.05$ vs diabetic non-Tg mice

phase in diabetic non-Tg mice, whereas renal function was well preserved in diabetic BNP-Tg mice.

Renal histology of diabetic mice We examined renal histological changes in diabetic non-Tg and BNP-Tg mice 16 weeks after induction of diabetes (Fig. 2). Compared with control (Fig. 2a), diabetic non-Tg mice showed



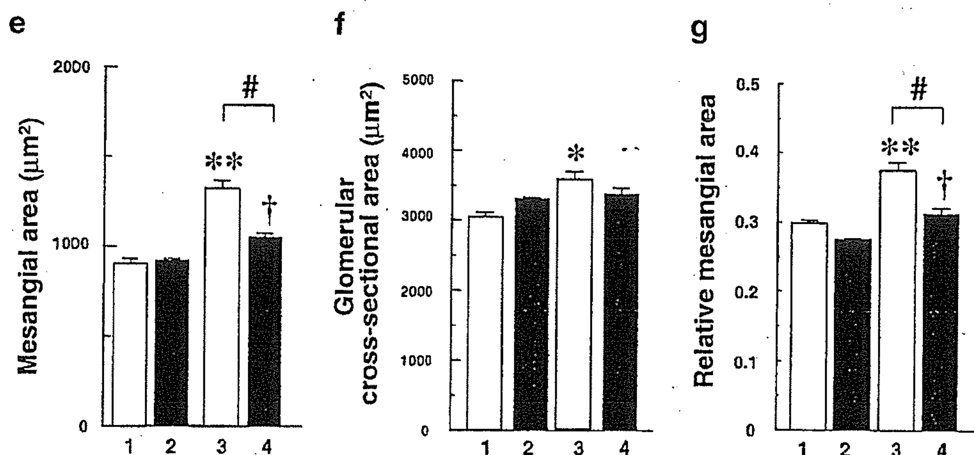


Fig. 2 Glomerular histology of diabetic mice at 16 weeks after induction of diabetes. Representative glomeruli from control non-Tg (**a**), control BNP-Tg (**b**), diabetic non-Tg (**c**), and diabetic BNP-Tg (**d**) mice are shown. Periodic acid–Schiff stain; magnification: ×400. Glomerular mesangial area (**e**), glomerular cross-sectional area (**f**) and relative mesangial area (**g**) in control and diabetic mice at 16 weeks. 1, control non-Tg mice (*n*=5); 2, control BNP-Tg mice (*n*=8); 3, diabetic non-Tg mice (*n*=10); 4, diabetic BNP-Tg mice (*n*=10). Values are expressed as means±SEM. *$p<0.05$, **$p<0.01$ vs control non-Tg mice; †$p<0.05$ vs control BNP-Tg mice; #$p<0.01$ vs diabetic non-Tg mice

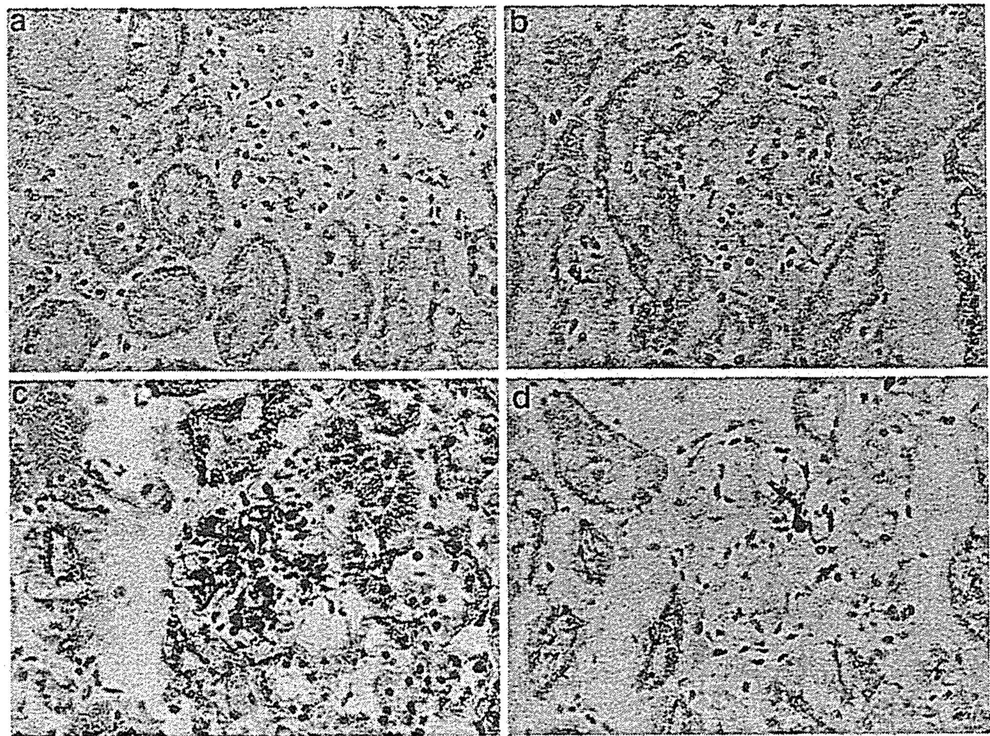


Fig. 3 Immunohistochemistry of TGF-β in control and diabetic mouse kidney at 16 weeks after induction of diabetes. Representative glomeruli from control non-Tg (**a**), control BNP-Tg (**b**), diabetic non-Tg (**c**), and diabetic BNP-Tg (**d**) mice are shown. Magnification: ×400

marked mesangial expansion and glomerular hypertrophy, along with partial tubular atrophy (Fig. 2c). In contrast, renal histology of diabetic BNP-Tg mice revealed only minimal glomerular hypertrophy and mesangial expansion with almost intact tubules (Fig. 2d), and was virtually indistinguishable from that of control BNP-Tg mice (Fig. 2b). Quantitative analysis revealed that the increase in mesangial area was marked in diabetic non-Tg mice, whereas this increase was significantly suppressed in diabetic BNP-Tg mice (1,328±58 vs. 1,037±21 μm^2, $p<0.01$, $n=10$ per group) (Fig. 2e). Glomerular hypertrophy was evident in diabetic non-Tg mice compared with control non-Tg mice (3,574±107 vs 3,048±66 μm^2, $p<0.05$), but was not apparent in BNP-Tg mice (3,378±103 vs 3,313± 30 μm^2 for diabetic vs control, respectively) (Fig. 2f). Accordingly, the increase in the relative mesangial area (mesangial area per glomerulus) was significantly blunted in diabetic BNP-Tg mice (Fig. 2g). Among the control mice, the mean glomerular area was slightly larger in the BNP-Tg mice than the non-Tg mice, but this difference was not statistically significant (Fig. 2f). These results indicate that the renal histological changes characteristic of diabetic nephropathy were prevented to a great extent in BNP-Tg mice.

Renal TGF-β1 expression The upregulation of TGF-β is postulated to play a pivotal role in facilitating extracellular matrix accumulation and subsequent glomerulosclerosis in diabetic glomerular injury [27, 28]. We therefore examined renal expression of the genes for TGF-β_1 (*Tgfb1*) and α1 (IV) collagen (*Col4a1*) in diabetic mice. By immunohistochemistry, we found that levels of TGF-β were markedly elevated in diabetic non-Tg mice (Fig. 3c) compared with control non-Tg mice (Fig. 3a), especially in the mesangial area. In diabetic BNP-Tg mice, on the other hand, this increase was significantly attenuated (Fig. 3d vs Fig. 3b). Likewise, northern blot analyses for *Tgfb1* and *Col4a1* revealed 3.5- to 4-fold increases in diabetic non-Tg mice compared with control ($p<0.05$, $n=7$) (Fig. 4a,b), whereas this upregulation was significantly reduced in diabetic BNP-Tg mice (1.3-fold increase relative to control BNP-Tg mice, $p<0.05$ vs diabetic non-Tg mice) (Fig. 4b). Thus, glomerular activation of the TGF-β-extracellular matrix protein cascade was significantly inhibited in diabetic BNP-Tg mice.

Renal ERK activation Accumulating evidence indicates that activation of the ERK/MAPK signalling pathway plays a key role in the induction of *Tgfb1* and extracellular matrix accumulation in diabetic nephropathy [29–31]. To address the mechanisms by which *Tgfb1* and matrix gene expression was inhibited in BNP-Tg mice, we investigated the phosphorylation of ERK in the kidney. Although ERK phosphorylation was minimal in the kidney tissues of both non-Tg and BNP-Tg mice under basal conditions, we found that levels of phosphorylated ERK were significantly increased in kidneys of diabetic mice (Fig. 5a). Importantly, phosphorylation of ERK in vivo was significantly lower in kidneys of diabetic BNP-Tg mice relative to diabetic non-Tg mice (Fig. 5b).

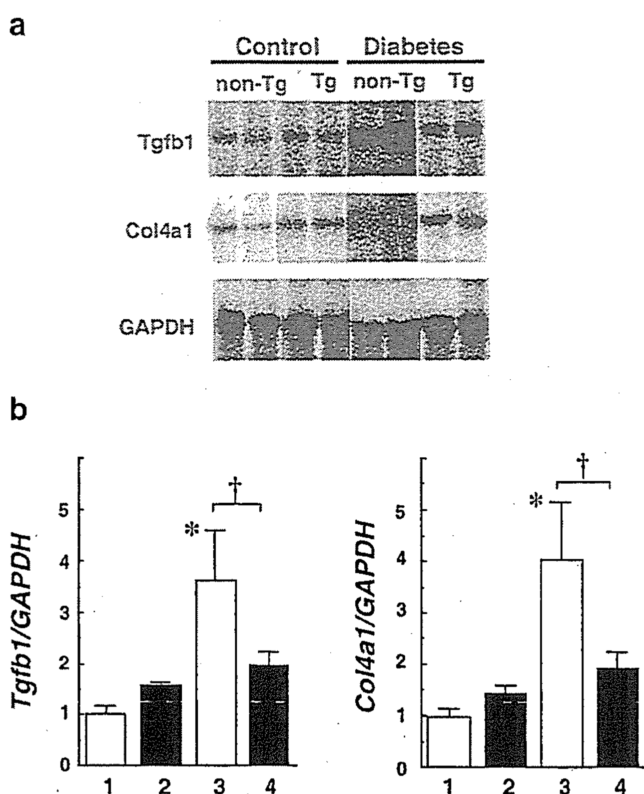


Fig. 4 Representative northern blots for *Tgfb1* and *Col4a1* mRNA expression (**a**) and their quantitative analysis (**b**) in kidney of control and diabetic mice at 16 weeks after induction of diabetes. 1, control non-Tg mice (n=4); 2, control BNP-Tg mice (n=4); 3, diabetic non-Tg mice (n=7); 4, diabetic BNP-Tg mice (n=7). Values are expressed as means±SEM. *p<0.05 vs control non-Tg mice. †p<0.05

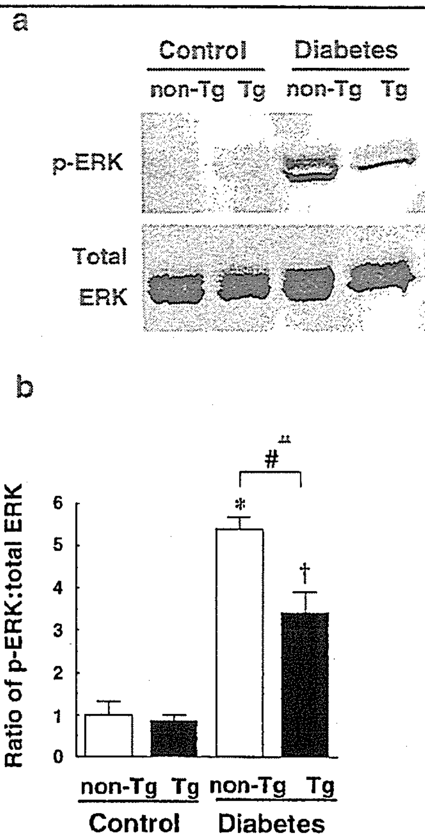


Fig. 5 ERK phosphorylation in the kidney of diabetic mice. Representative western blots of phosphorylated ERK-1/2 and total ERK-1/2 at 16 weeks after induction of diabetes (**a**) and quantitative analysis of phospho-ERK:total ERK (**b**). Values are expressed as means±SEM for n=4 in each group. *p<0.01 vs control non-Tg mice; †p<0.01 vs control BNP-Tg mice. #p<0.01

Effects of hydralazine administration Analyses thus far have suggested that a chronic excess of BNP prevents the progression of diabetic renal injury. Systemic blood pressure reduction is crucial to retard the progression of renal and vascular complications in diabetes [6, 32]. In order to explore whether the beneficial effects observed in BNP-Tg mice were the result of systemic hypotension, we studied the effect of hydralazine administration in diabetic non-Tg mice. Despite an effective reduction in systemic blood pressure to a level comparable to that in diabetic BNP-Tg mice (Fig. 6a), this treatment failed to prevent the increase in albuminuria (Fig. 6b) or to alleviate renal histological changes (data not shown). These results indicate that systemic hypotension in BNP-Tg mice does not play an important role in the renoprotective effects observed.

Effects of BNP in cultured mesangial cells under high glucose We next addressed the direct actions exerted by BNP using cultured mesangial cells under high glucose conditions. First, we investigated the effects of BNP on the expression of *Tgfb1* and the gene encoding its downstream effector, fibronectin. High glucose conditions significantly augmented *Tgfb1* and *Fn1* mRNA expression (1.8-fold and 1.5-fold of control [normal glucose, no BNP], respectively) (Fig. 7a,b). This effect was not simply due to high osmotic conditions, because an osmotic control with mannitol had no effect on *Tgfb1* mRNA expression (1.7-fold of control with high glucose vs 1.1-fold with mannitol, p<0.05) (Fig. 7c). This upregulation of *Tgfb1* and *Fn1* under high glucose conditions was effectively abolished by the addition of BNP (1.2-fold and 1.0-fold of control, respectively, p<0.01), or with a membrane-permeable analogue of cGMP (1.1-fold and 0.9-fold of control, respectively, p<0.01) (Fig. 7a,b).

We further examined the effects of BNP and cGMP on ERK phosphorylation of mesangial cells under high glucose conditions. High glucose-induced ERK phosphorylation in mesangial cells was significantly inhibited by the addition of BNP or cGMP (Fig. 8a). Moreover, BNP and cGMP effectively prevented ERK phosphorylation induced by PKC activation with PMA (Fig. 8b). Taken together, these results suggest that BNP inhibited PKC-ERK pathway activation and subsequent matrix gene activation, at least in part, locally in the mesangium in vivo, thereby leading to the amelioration of diabetic renal injury.

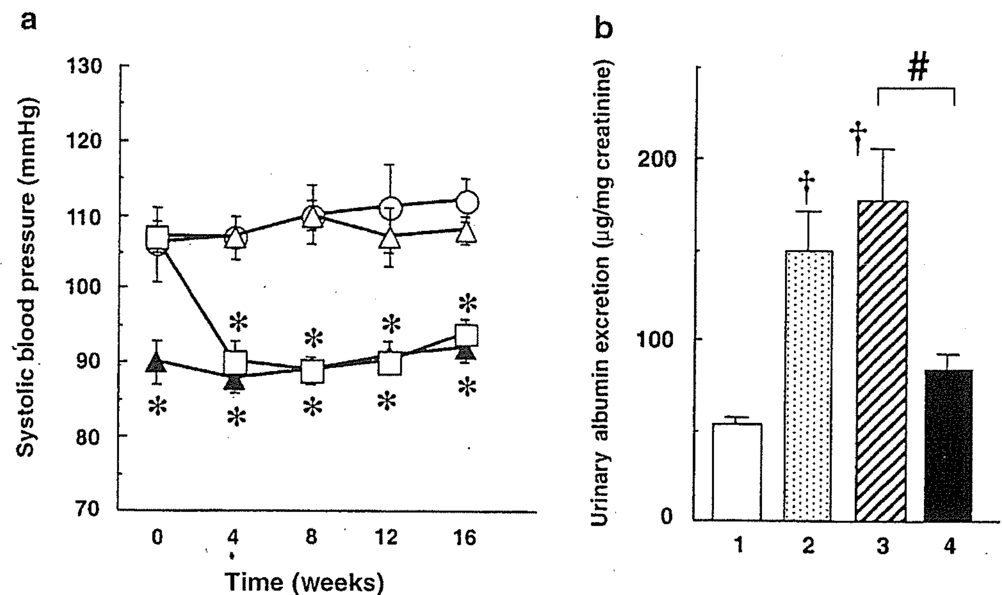


Fig. 6 **a** Effects of hydralazine administration on systolic blood pressure. *Open circles*, control non-Tg mice (n=5); *open triangles*, diabetic non-Tg mice (n=10); *closed triangles*, diabetic BNP-Tg mice (n=10); *open squares*, hydralazine-treated diabetic non-Tg mice (n=7). *p<0.005 vs diabetic non-Tg mice. **b** Urinary albumin excretion normalised to creatinine at 16 weeks. 1, control non-Tg mice; 2, diabetic non-Tg mice; 3, hydralazine-treated diabetic non-Tg mice; 4, diabetic BNP-Tg mice. $^{\dagger}p$<0.02 vs control non-Tg mice; $^{\#}p$<0.05

Discussion

Previous reports have suggested that increased natriuretic peptides may affect the course of diabetic nephropathy. Acute inhibition of elevated plasma ANP decreased hyperfiltration and albuminuria in experimental models [18, 19]. In patients with early-stage type 1 diabetes, ANP concentration was correlated with glomerular filtration rate [33]. Furthermore, acute ANP infusion increased the glomerular filtration rate, filtration fraction and albuminuria [34]. These studies investigated early haemodynamic abnormalities or only short-term effects of ANP (within several hours). Thus, the aim of this study was to assess the long-term effects of natriuretic peptides on glomerular function and histology. In order to address these issues, we investigated the effects of a chronic excess of BNP on the progression of diabetic nephropathy using BNP-Tg mice.

In this study, we demonstrate that transgenic overexpression of BNP prevents the progression of diabetic nephropathy in mice. In diabetic BNP-Tg mice, the accumulation of mesangial matrix was only minimal, with virtually no glomerular hypertrophy, in contrast to the histology of diabetic non-Tg mice (Fig. 2). Consistent with such histological amelioration, albuminuria was significantly attenuated in BNP-Tg mice, and renal function was well preserved (Fig. 1, Table 2). Although absolute creatinine values may be less accurate than those measured by HPLC, assessment of renal function in these groups should be valid considering the changes in serum creatinine essentially paralleled those in urea nitrogen (Table 2). These findings provide the first evidence that a chronic excess of BNP prevents the kidney from developing diabetic renal injury. We also demonstrated that the upregulation of *Tgfb* expression and protein levels in diabetic kidneys were markedly inhibited in BNP-Tg mice (Figs 3, 4). Considering a pathogenic role for TGF-β in cellular dysfunction, fibrogenesis and glomerular hypertrophy in diabetes [3, 28, 35], it is conceivable that the inhibition of renal TGF-β system activation contributed significantly to the observed protective effects of BNP from diabetic renal injury. Although the effect of BNP on glomerular haemodynamics was not investigated, it may be possible that the chronic excess of BNP lessened glomerular hypertension by inhibiting the RAS and TGF-β system tonically. It has been shown that combined inhibition of both angiotensin-converting enzyme and neutral endopeptidase, which potentiates ANP and BNP, results in lower glomerular capillary pressure than the former alone in subtotally nephrectomised rats [36].

There is no doubt that tight blood pressure control is important for retarding or preventing the progression of diabetic renal injury [6, 32]. Clearly, the diabetic BNP-Tg mice showed lower blood pressure. Although systemic blood pressure reduction with hydralazine treatment failed to alleviate nephropathy in diabetic non-Tg mice (Fig. 6), precise blood pressure profiles should have been different between the two hypotensive groups. We therefore cannot exclude the possibility that low blood pressure could account for the observed renoprotective effect in BNP-Tg mice.

ERK plays a pivotal role in activating mesangial TGF-β expression/signalling and extracellular matrix accumulation [29, 30]. We clearly show that ERK activation in the kidney tissue was significantly attenuated in BNP-Tg mice (Fig. 5).

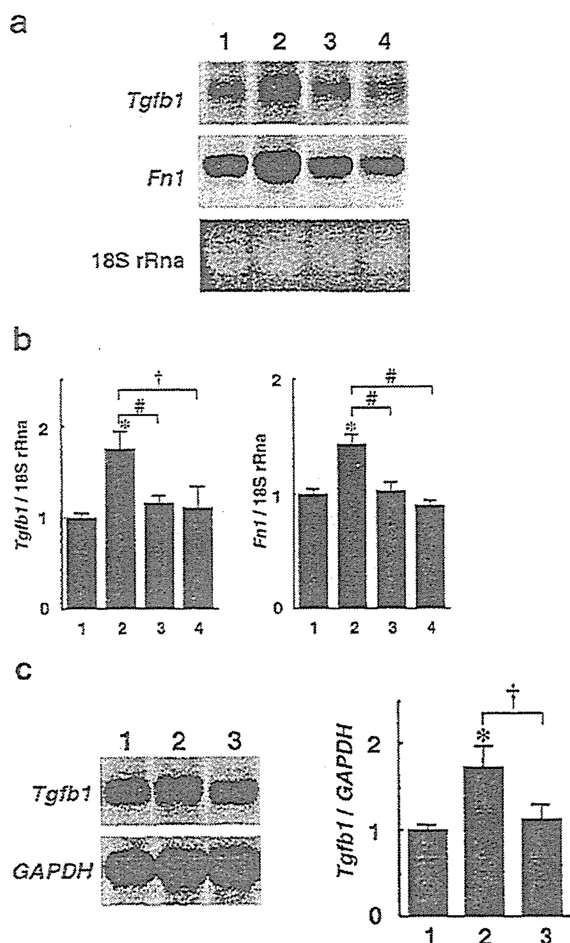


Fig. 7 Representative northern blots for *Tgfb1* and *Fn1* mRNA expression (a) and their quantitative analysis (b) in cultured mesangial cells under high glucose conditions. 1, normal glucose (5.6 mmol/l); 2, high glucose (25 mmol/l); 3, high glucose+BNP 100 nmol/l; 4, high glucose+cGMP 1 mmol/l. Values are expressed as means±SEM for n=6 in each group. *p<0.01 vs normal glucose; †p<0.02; #p<0.01. c. Representative northern blots for *Tgfb1* mRNA expression and quantitative analysis in cultured mesangial cells. 1, normal glucose (5.6 mmol/l); 2, high glucose (25 mmol/l); 3, normal glucose+mannitol (19.4 mmol/l). Values are expressed as means±SEM for n=4 for each group. *p<0.02 vs normal glucose. †p<0.05

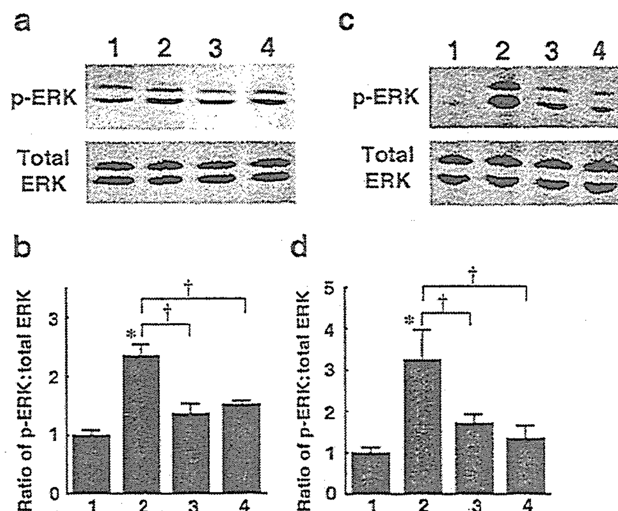


Fig. 8 a, b Inhibitory effects of BNP on ERK phosphorylation in cultured mesangial cells under high glucose conditions. 1, normal glucose (5.6 mmol/l); 2, high glucose (25 mmol/l); 3, high glucose+BNP 100 nmol/l; 4, high glucose+cGMP 1 mmol/l. Values are expressed as means±SEM for n=4 in each group. *p<0.01 vs normal glucose; †p<0.01. c, d. Inhibitory effects of BNP on PMA-induced ERK phosphorylation. 1, control; 2, PMA 100 nmol/l; 3, PMA 100 nmol/l+BNP 100 nmol/l; 4, PMA 100 nmol/l+cGMP 0.1 mmol/l. Values are expressed as means±SEM for n=4 in each group. *p<0.01 vs control; †p<0.01

We also demonstrated that BNP effectively inhibited ERK phosphorylation, as well as TGF-β expression, in cultured mesangial cells under high glucose conditions (Figs 7, 8). High glucose conditions activate PKC, and ERK activation occurs through a PKC-dependent mechanism [31, 37]. BNP also attenuated PKC-induced phosphorylation of ERK. It has been reported that ANP inhibits MAPK activation downstream of PKC via MAPK phosphatase activation in a cGMP-dependent manner [37]. These results therefore suggest that BNP exerts renoprotective effects in diabetes at least partly by locally inhibiting activation of the PKC-ERK pathway at the mesangium. The pathogenic role of PKC in matrix gene activation, haemodynamic abnormalities and proteinuria in vivo has already been shown in diabetic rodent models using a PKC β inhibitor [38, 39].

Podocytes are highlighted as the key in maintaining normal glomerular function and structure [40, 41], and podocyte loss or injury is closely associated with diabetic glomerular injury [42]. Natriuretic peptides act on podocytes and may modulate their function [43], but how natriuretic peptides affect podocytes in diabetic states or whether BNP exerted beneficial effects against podocyte injury in the current study are unclear. Further studies are needed to explore the effects of BNP on podocytes during the course of diabetic nephropathy.

The effect of BNP on glycaemic control is another issue to be addressed. Natriuretic peptides generally act to antagonise the systemic and local actions of angiotensin II [9]; they are therefore considered as endogenous RAS inhibitors. Growing evidence suggests that inhibition of the RAS exerts a beneficial effect on glycaemic control in experimental models and in clinical studies [44–46]. In the present study, we found no significant difference in blood glucose levels between non-Tg and BNP-Tg mice in this type of insulin-deficient model (Table 1). Whether natriuretic peptides or agonists of this system are beneficial in terms of regulating glycaemic control and preventing diabetic complications requires further investigation.

We have previously reported that BNP-Tg mice with higher copy numbers of the transgene show marked skeletal overgrowth [47], indicating that BNP likely activates the

physiological CNP/GC-B pathway in the bone to stimulate endochondral ossification [48]. Therefore, it is important to clarify whether the beneficial effects of BNP observed in this study are GC-A-dependent or GC-B-dependent. In the kidney, GC-A is localised in the mesangium, capillary and tubules, whereas GC-B is localised in the tubular system [11]. In the present study, BNP inhibited glomerular ERK activation and *Tgfb1* expression, suggesting that the effects of BNP were mediated via GC-A. However, it should be noted that the cultured mesangial cells used in our study express both GC-A and GC-B, where CNP has potent antifibrotic effects [24]; therefore the observed in vitro effects of BNP might also be exerted via GC-B. Analyses of crosses between BNP-Tg mice and GC-A null mice [10] and other combinations would answer these questions.

In summary, we demonstrate that a chronic excess of BNP in mice prevents diabetic glomerular injury, with amelioration of albuminuria and renal dysfunction, and these effects may be beyond those from mere systemic blood pressure reduction. Although we need to be cautious in interpreting these results and extrapolating them to clinical situations, our study opens up the possibility that the renoprotective effects of natriuretic peptides may be therapeutically applicable for the prevention of progression of diabetic nephropathy.

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