Ameliorated Glomerular Injury in Mice Overexpressing Brain Natriuretic Peptide with Renal Ablation

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Abstract. Brain natriuretic peptide (BNP) is a cardiac hormone produced by the ventricle, and its secretion is markedly increased in heart failure, hypertension, and renal failure. Transgenic mice that overexpress BNP in the liver (BNP-Tg) were recently generated, resulting in low BP. To elucidate the role of BNP in renal pathophysiology, the effect of chronic excess of BNP in transgenic mice on glomerular injury after subtotal nephrectomy induced by resection of the renal poles was examined. After nephrectomy, glomerular cross-sectional areas in control nontransgenic mice markedly increased as compared with those in sham-operated mice (+81 ± 7%), whereas there was only a modest increase in BNP-Tg (+10 ± 6%). Expansion of the mesangial area and increase in the intraglomerular cell number were also inhibited in BNP-Tg. Glomerular expressions of transforming growth factor-β and fibronectin were increased with hypertrophy and were significantly suppressed in BNP-Tg. Furthermore, increases in the urinary albumin excretion and BP were significantly ameliorated in BNP-Tg. Chronic hydralazine treatment in nephrectomized nontransgenic mice failed to inhibit glomerular hypertrophy. These findings indicate that the chronic excess of BNP in mice ameliorates glomerular hypertrophy and mesangial expansion after renal ablation. The results also suggest that the observed effects of natriuretic peptides under reduced renal mass are not due merely to systemic BP reduction and may be therapeutically applicable in various renal diseases.

Cardiovascular homeostasis and renal function are maintained through complex interactions among multiple regulatory factors. Of these factors, a family of natriuretic peptides consisting of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) exert a variety of biologic actions such as diuresis, natriuresis, vasorelaxation, and inhibition of renin and aldosterone secretion, thereby participating in body fluid homeostasis and BP control as circulating hormones and as neuropeptides (1–6). ANP is synthesized and secreted predominantly by the cardiac atria in response to atrial stretch (1). In patients with congestive heart failure, ANP secretion is augmented in relation to the severity of heart failure (7), along with increased contribution of ventricular synthesis and secretion of ANP (8). BNP was originally isolated from the porcine brain and hence thought to act as a neuropeptide (5). Subsequently, we have demonstrated that BNP is a cardiac hormone in humans and rodents that is produced mainly by the ventricles (1,9). Secretion and plasma levels of BNP are markedly increased and often exceed the levels of ANP in patients and in animal models with congestive heart failure, myocardial infarction, hypertension, and renal failure (1,9–11). Furthermore, administration of BNP in patients with heart failure (12) exhibited potent renal and vascular effects with improved left ventricular function comparable to those observed in ANP infusion (13), providing the evidence that the augmented cardiac natriuretic peptides should represent one of the compensatory mechanisms for cardiorenal homeostasis in these disease states. CNP occurs in a wide variety of tissues, including brain (6), vascular endothelium (14), macrophages (15), and renal glomeruli and tubules (16), where it acts as a neuropeptide or as a local regulator.

Subtotal renal ablation results in hypertension, proteinuria, progressive glomerular hypertrophy, and subsequent renal failure (17). Factors that are responsible for this process are not clearly understood, but accumulating evidence suggests a role for increased intraglomerular pressure concomitant with adaptive glomerular hyperfiltration in causing the glomerular sclerosis in this model (17–19). Among the factors, the plasma level of ANP is increased in this model and the specific blockade of ANP or its receptor has resulted in reduction in the adaptive increase of sodium excretion (20,21), suggesting that endogenous ANP may have a role in promoting the renal adaptation of sodium and water excretion. Moreover, acute infusion of ANP in rats was shown to cause afferent arteriolar vasodilation with efferent arteriolar vasoconstriction (22). These observations suggest that hypersecretion of endogenous natriuretic peptides in this model may aggravate hyperfiltration and subsequent glomerular injury. In contrast, ANP infusion could exhibit beneficial effects in animal models and in pa-
tients with some forms of acute renal failure (23,24). Previous studies, however, gave no definite evidence for the pathophysologic role of chronic excess of natriuretic peptides in progression of chronic renal insufficiency that occurs under reduced renal mass.

Recently, we generated transgenic mice (Tg) that overexpress the mouse BNP gene in the liver (25). These mice (BNP-Tg) showed a 10- to 100-fold increase in plasma BNP levels accompanied by elevated plasma guanosine 3′,5′-cyclic monophosphate (cGMP) concentrations and exhibited significant reduction in arterial BP. BNP, sharing receptors with ANP, is thought to exert its actions mainly by activating a particulate guanylyl cyclase (GC)-coupled receptor, GC-A (2,26). Indeed, BNP-Tg seemed to share most phenotypes with those observed in ANP-Tg previously reported (27), suggesting that these animals could provide a model for chronic GC-A activation. In the present study, to elucidate the role of chronic excess of natriuretic peptides in renal pathophysiology and to explore the possibility of therapeutic application of natriuretic peptides in chronic renal disease, we investigated the effect of BNP overproduced in BNP-Tg on glomerular injury after subtotal nephrectomy.

Materials and Methods

Animals

All animal experiments were conducted in accordance with our institutional guidelines for animal research. Generation of several lines of BNP-Tg mice, harboring 15 to 50 copies of the transgene that are under the control of the human serum amyloid P component promoter, was reported previously (25). This promoter was active only in the liver after birth (25). In this study, BNP-Tg (line 55) with approximately 20 copies of the transgene and their control littermates, nontransgenic C57BL/6J mice (non-Tg), were used at 12 to 15 wk of age at the start of the study. This line of BNP-Tg showed a marked increase in plasma BNP levels (1.8 ± 1.1 pmol/ml), as already reported (28). Mice were kept in our laboratory for 7 d before the first procedure, fed on standard mouse chow (0.5% NaCl; Japan Clea, Tokyo, Japan), and given water ad libitum. We maintained these animals under alternating 12-h cycles of light and dark.

Renal Ablation

Subtotal renal ablation was performed by the surgical excision method (29). All surgical procedures were carried out under anesthesia with intraperitoneal pentobarbital (30 mg/kg body wt; Sigma, St. Louis, MO). The left kidney was exposed through a left paramedian incision and decapsulated, leaving the adrenal gland intact. The upper and lower poles (two thirds of the left kidney) were resected, and the remaining kidney was allowed to recover for 1 wk. Then the remaining right kidney was removed through a right paramedian incision after ligation of the right renal artery, vein, and ureter. Sham-operated mice were subjected to brief ligation of the renal artery, vein, and ureter, and the incision was closed without removal.

BP Measurement

The systolic, mean, and diastolic BP were measured every 4 wk by a programmable sphygmomanometer (BP-98A; Softron, Tokyo, Japan) using the tail-cuff method (30). At least six readings were taken for each measurement. For a hydralazine administration group, nephrectomized non-Tg mice (n = 5) were given the drinking water containing 60 mg/L of hydralazine hydrochloride (Sigma) ad libitum from 1 wk after the completion of nephrectomy over 15 wk, and BP was measured every 3 wk.

Blood and Urine Physiologic Measurements

Blood samples were obtained from the retro-orbital sinus from these mice before and 16 wk after nephrectomy, and serum creatinine levels were measured (31). For urine measurements, each mouse was separately housed in a metabolic cage (Shinano Manufacturing, Tokyo, Japan), and the daily urine volume and urinary creatinine excretion were measured (31). Urinary albumin excretion was assayed with a murine albumin enzyme-linked immunosorbent assay kit (Exocell, Philadelphia, PA). To standardize urinary albumin excretion for GFR, albuminuria was expressed as micrograms of urinary albumin per milligram of urinary creatinine.

Histology and Morphometric Analysis

For light microscopy, sagittal kidney sections were fixed by immersion in Carnoy’s solution followed by 4% buffered formaldehyde and embedded in paraffin. Two-μm-thick sections were stained with hematoxylin and eosin or with periodic acid-Schiff (PAS) stain. The glomerular cross-sectional area was measured quantitatively with a computer-aided manipulator (KS400; Carl Zeiss Vision, Munich, Germany) by tracing the outer margin of the glomerular tuft (32). Intraglomerular cell number was measured by counting the nuclei within the glomerular tuft. More than 20 consecutive glomerular sections randomly selected in each mouse by scanning from the outer cortex to the corticomedullary junction were examined by two investigators without knowledge of the origin of the slides. After knowing that variations between the observers were within 10%, the mean cross-sectional area and the cell number were calculated. PAS-stained areas in the glomeruli were also calculated and regarded as the mesangial areas (32).

Immunohistochemistry

To examine the expressions of transforming growth factor-β (TGF-β) and fibronectin, immunohistochemical studies were performed using the avidin-biotin-peroxidase complex technique (33) (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA). The kidney tissues were embedded in OCT compound (Sakura Fine-technical, Tokyo, Japan) and snap-frozen in liquid nitrogen. Cryostat sections (4-μm thick) were washed with phosphate-buffered saline (PBS) and treated with 0.3% H2O2 in PBS for 20 min to quench endogenous peroxidase activity. After wash and blocking with 1.5% normal goat serum, the specimens were incubated overnight at 4°C with primary antibodies appropriately diluted in 1% BSA/PBS: rabbit polyclonal anti-TGF-β1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal antifibronectin antibody (DAKO, Glostrup, Denmark). After being rinsed with 1% BSA/PBS, the sections were incubated with biotinylated anti-rabbit Ig for 2 h, then with the avidin-biotin-peroxidase complex, and finally developed with 3,3′-diaminobenzidine tetrahydrochloride (Kanto Chemical, Tokyo, Japan). Nonimmune rabbit serum was used as negative control and gave no significant staining. In these experiments, the slides were read in a blinded fashion and graded semiquantitatively (0 to 3+) according to the degree of positive staining as described (33,34) with slight modification (0 = none or <5%, 1+ = 5 to 25%, 2+ = 25 to 50%, and 3+ = >50%), and the mean score per section was calculated.
Statistical Analyses

Results were expressed as the mean ± SEM. Data were analyzed by t test or by ANOVA followed by Scheffe’s test. P < 0.05 was considered statistically significant.

Results

Baseline Characteristics of BNP-Tg Mice

Table 1 shows the baseline characteristics of BNP-Tg (line 55) and control non-Tg used in this study. Tail-cuff BP of BNP-Tg were significantly reduced, by approximately 15 mmHg lower than those of non-Tg, consistent with a previous report (25). The differences in heart rate, body weight, and kidney weight were insignificant. BNP-Tg tended to exhibit an increase in urine volume, although the difference did not achieve statistical significance.

Histology and Morphometric Analysis

Histologic examination of the kidneys from sham-operated mice revealed no appreciable differences in the glomeruli or tubules between non-Tg and BNP-Tg (Figure 1, a and b, at low magnification and Figure 1, e and f, at higher magnification). Sixteen wk after ablation, kidney sections from non-Tg revealed a remarkable glomerular hypertrophy and increased intraglomerular cells mostly in the mesangial area with mesangial expansion (Figure 1, c and g). Moderate tubular enlargement was also noted, with minimal signs of interstitial inflammation or fibrosis. In BNP-Tg, however, there were only modest increases in the mesangial matrix and intraglomerular cells, with no apparent signs of glomerular hypertrophy or tubulointerstitial injury (Figure 1, d and h).

To compare these changes more quantitatively, the glomerular cross-sectional area and intraglomerular cell number of every specimen were analyzed (Figure 2). The average cross-sectional area of the glomerular tuft of BNP-Tg was similar to that of non-Tg in sham-operated mice (2.96 ± 0.14 × 10⁻³ mm² versus 2.68 ± 0.14 × 10⁻³ mm²). Glomerular cross-sectional areas of non-Tg were markedly increased 16 wk after ablation (+81.0 ± 7.4%, P < 0.01 compared with sham-operated non-Tg), but interestingly, this increase was significantly suppressed in BNP-Tg (+10.1 ± 5.7%; Figure 2a). The glomerular hypertrophy was accompanied by a significant expansion of the mesangial areas in non-Tg (+64.7 ± 16.1%, P < 0.05 compared with sham-operated non-Tg), but there was no significant change in BNP-Tg (+19.3 ± 16.3%). Furthermore, the increase in the cell number within the glomerular tuft, which occurred mostly in mesangial cells, was observed in non-Tg but was not as evident in BNP-Tg (Figure 2b). These results indicate that the histologic changes associated with glomerular adaptive hypertrophy of the remnant kidney after subtotal nephrectomy were significantly prevented in BNP-Tg.

Immunohistochemical Analysis of Glomerular TGF-β and Fibronectin Expressions

To evaluate further the possible phenotypic changes of the glomeruli, we analyzed by immunohistochemistry the expressions of TGF-β1 and fibronectin, which are regarded as key molecules that lead to glomerulosclerosis and renal fibrosis after renal ablation (35) (Figure 3). Sham-operated mice showed a trivial glomerular staining of TGF-β1 in both non-Tg and BNP-Tg (Figure 3, a and b). Sixteen wk after nephrectomy, the glomerular expression of TGF-β1 was significantly augmented in non-Tg (Figure 3c), whereas this upregulation was very mild in BNP-Tg (Figure 3d). Similarly, staining of fibronectin showed low glomerular expression at baseline and in sham-operated controls (not shown); glomerular staining of fibronectin was increased significantly in nephrectomized non-Tg (Figure 3e), whereas it was mild in BNP-Tg (Figure 3f). Semiquantitative analyses of glomerular staining of TGF-β1 and fibronectin revealed a significant suppression of this upregulation after nephrectomy in BNP-Tg as compared with non-Tg (Figure 4).

Changes in Blood and Urine Parameters

To evaluate the functional alterations after subtotal nephrectomy in these mice, we measured serum creatinine levels, urine volume, and urinary creatinine and albumin excretions (Figure 5, Table 2). The basal serum creatinine level was normal in BNP-Tg (0.16 ± 0.01 mg/dl) and was comparable to that in the control non-Tg (0.14 ± 0.01 mg/dl; Figure 5a). The baseline urinary creatinine excretion and the endogenous creatinine clearance were also not significantly different between non-Tg and BNP-Tg (Table 2).

In non-Tg, a significant increase in the urine volume (3.5 times greater than the baseline level) was observed at 5 wk after nephrectomy with a relatively high level of urinary creatinine excretion (Table 2). Increase in the urine volume was still present at 8 wk (2.1 times) after ablation. BNP-Tg tended to have more urine output at the basal level but showed less pronounced increase in the urine volume after nephrectomy, with 1.9 times of the baseline level at 5 wk (Table 2). The urine volume returned to the baseline level within 8 wk after ablation.

Table 1. Baseline characteristics of BNP-transgenic mice (BNP-Tg) and control nontransgenic mice (non-Tg)*

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<td>Urine volume (ml/24 h)</td>
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<td>1.52 ± 0.24</td>
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*Values are expressed as mean ± SEM. Blood pressure values were measured by the tail-cuff method as outlined in Materials and Methods.

b P < 0.01 compared with non-Tg.
Figure 1. Histology of the kidneys at low magnification (A through D, ×100) and higher magnification (E through H, ×400) from non-transgenic (non-Tg) and brain natriuretic peptide–transgenic mice (BNP-Tg) after subtotal nephrectomy. Representative periodic acid-Schiff (PAS)-stained sections from non-Tg (A, C, E, and G) and BNP-Tg (B, D, F, and H) 16 wk after sham operation (A, B, E, and F) or five sixths renal ablation (C, D, G, and H) are shown. Kidney histology of the sham-operated mice revealed no remarkable differences in the glomeruli or tubules between non-Tg and BNP-Tg (A and B, and E and F). Sixteen wk after ablation, glomeruli from non-Tg exhibited a remarkable hypertrophy with mesangial expansion and increased intraglomerular cells (C and G). In contrast, there were only modest increases in the mesangial matrix and intraglomerular cells, with no apparent signs of glomerular hypertrophy in BNP-Tg (D and H).
Changes in BP

The tail-cuff BP in control non-Tg tended to increase within 2 to 4 wk after nephrectomy and showed a slight but significant increase at 8 wk as compared with basal levels (systolic, 107.0 ± 2.3 mmHg versus 99.4 ± 2.4 mmHg, P < 0.01; diastolic, 78.2 ± 2.2 mmHg versus 72.4 ± 2.1 mmHg, P = 0.06; n = 6). Significant systemic hypertension persisted at 16 wk in these mice (systolic, 113.0 ± 1.1 mmHg, P < 0.01; diastolic, 78.4 ± 2.7 mmHg, P < 0.05, as compared with basal levels, n = 6). In contrast, BNP-Tg showed no significant changes in BP after nephrectomy and remained hypotensive even 16 wk after ablation (systolic, 86.6 ± 2.4 mmHg versus 85.4 ± 2.6 mmHg; diastolic, 61.5 ± 2.1 mmHg versus 61.2 ± 3.3 mmHg; n = 6). There were no significant BP changes in sham-operated mice throughout the observation period (data not shown).

Effects of Hydralazine Administration

Analyses have indicated that the chronic excess of BNP inhibits the increase in the BP and prevents the histologic changes of adaptive glomerular hypertrophy in this renal ablation model. To explore whether the latter effect was due to systemic BP reduction, we examined the effect of hydralazine administration in nephrectomized non-Tg. In spite of effective reduction in the BP to the level comparable to that of BNP-Tg (Figure 6a), this treatment failed to inhibit glomerular hypertrophy (4.35 ± 0.25 × 10^-3 mm^2) (Figure 6b). Similarly, there was no significant improvement in the glomerular hypercellularity, mesangial expansion, or glomerular expressions of TGF-β and fibronectin (data not shown). These results suggest that the systemic BP reduction in BNP-Tg may not play a critical role in these effects on the remnant kidney.

Discussion

In the present study, we investigated the effect of chronic excess of BNP overproduced systemically in BNP-Tg mice on histologic and functional alterations of the kidney in the renal ablation model. This experimental model has been widely used in rats to investigate the mechanisms of the progression of renal injury under reduced nephron number, resulting in hypertension, proteinuria, glomerular hypertrophy and sclerosis, and interstitial fibrosis with subsequent renal failure (17–19,34,35). We demonstrate that, in mice, the control animals (non-Tg) with this procedure exhibited mild to moderate systemic hypertension, increased serum creatinine levels, and albuminuria together with remarkable glomerular hypertrophy and moderate mesangial expansion 16 wk after ablation.

The present study clearly demonstrates that the glomerular morphologic changes in the remnant kidney were significantly suppressed in BNP-Tg (Figures 1 and 2). The glomerular size measured as the cross-sectional area almost doubled in the control mice 16 wk after nephrectomy, whereas no appreciable change in size was observed in BNP-Tg. Concomitant increases in the mesangial area and intraglomerular cell number were also lessened in BNP-Tg. Glomerular hypertrophy with mesangial expansion is a characteristic feature in various experimental conditions, including subtotal nephrectomy (17–19), diabetes, and dietary protein excess (36), and also in multiple clinical situations, such as focal segmental glomerulosclerosis and diabetic nephropathy (36), which may eventually lead to glomerular sclerosis. The regulation of the glomerular volume in these models is not fully clarified, but glomerular hypertension, resulting from hyperperfusion in the residual glomeruli with increased single nephron GFR, is thought to be the major determinant (19,36). In the present study, urine measurements after nephrectomy (Table 2) revealed more pronounced diuresis with relatively higher creatinine excretion (90% instead of one sixth of the basal at 5 wk) in non-Tg. This finding may suggest that compensatory hyperfiltration occurred in the control mice during this early period after nephrectomy. Because the direct measurement of the
intraglomerular pressure or single nephron GFR was not performed in the present study, whether the inhibition of glomerular hypertrophy in BNP-Tg resulted from lower intraglomerular pressure or less hyperfiltration in the remnant nephron during the early period should await further clarification.

Multiple humoral factors have been identified as possible mechanisms by which glomerular hypertension with hypertrophy leads to glomerulosclerosis and to subsequent deterioration in GFR. Among others, the activation of the renin-angiotensin system is believed to be a key step (35), as evidenced by the effects of the interruption of this system resulting in profound structural and functional amelioration (19,37). Of note, the activation of the renin-angiotensin system is thought to occur locally in the kidney, with its circulating counterpart un-

**Figure 3.** Immunohistochemical analyses for TGF-β1 (A through D) and fibronectin (E and F) in non-Tg and BNP-Tg at 16 wk after subtotal nephrectomy. Representative results of immunoperoxidase staining from non-Tg (A, C, and E) and BNP-Tg (B, D, and F) 16 wk after sham operation (A and B) or nephrectomy (C through F) are shown. Sham-operated mice showed a trivial glomerular staining of TGF-β1 in both non-Tg (A) and BNP-Tg (B). After nephrectomy, the glomerular expression of TGF-β1 was augmented mainly in the mesangial area in non-Tg (C), whereas this upregulation was very mild in BNP-Tg (D). Similarly, glomerular staining of fibronectin was increased significantly in nephrectomized non-Tg (E), whereas it was mild in BNP-Tg (F). Magnification, ×400.
excretion normalized with creatinine revealed a significant increase in non-Tg but not in BNP-Tg. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 6.

Figure 5. Changes in serum creatinine levels (a) and albuminuria (b) in non-Tg and BNP-Tg after subtotal nephrectomy. (a) There was no significant difference in basal serum creatinine levels between non-Tg and BNP-Tg. Sixteen wk after nephrectomy, the serum creatinine level increased significantly in non-Tg but not in BNP-Tg. Nx, subtotal nephrectomy. Data are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, n = 6–7. (b) At 16 wk, the urinary albumin excretion normalized with creatinine revealed a significant increase only in non-Tg. Data are expressed as the mean ± SEM. *P < 0.05, n = 6–10.

changed, or rather suppressed, as a result of systemic hypervolemia (35). After this activation, the stimulation of the downstream cascade involving multiple growth factors such as platelet-derived growth factor (34) and TGF-β (35) has been proposed to play a pivotal role in disease progression. The mechanisms that initiate the cascade of growth factor activation are not well understood but may be dependent on the renin-angiotensin system (35,38,39) as well as be direct effects of mechanical factors caused by altered hemodynamic forces (35,36).

The present study demonstrates that the chronic BNP excess inhibited the induction of TGF-β and fibronectin in the glomeruli after renal ablation (Figures 3 and 4). The role of TGF-β in the regulation of renal response to injury and in renal pathogenesis has been studied extensively (35) and has been implicated in several models of experimental renal injury and in human diseases (33,35,40,41). On the basis of these findings, TGF-β is nominated as one likely candidate for major therapeutic targets in renal diseases (40,42). The precise mechanisms of how the chronic excess of BNP inhibited glomerular TGF-β and fibronectin expressions in the present study remain to be elucidated, but several factors involving hemodynamic changes as well as direct effects on the intrarenal renin angiotensin-TGF-β axis are being considered. In general, natriuretic peptides act to antagonize not only systemic but also local actions of the renin-angiotensin system in a cGMP-dependent manner (1). Indeed, ANP inhibited the mesangial cell contraction induced by angiotensin II (43), the proliferation of mesangial cells (44), and the hypertrophic response of vascular smooth muscle cells to angiotensin II (45). Furthermore, ANP suppressed the mitogen-activated protein (MAP) kinase activity in mesangial cells (46), the activation of which could be a critical mediator of TGF-β induction (47). These actions reported for ANP are likely to be reproduced by BNP, because BNP and ANP have similar affinity to known GC-coupled receptors in the mesangium and vasculature (26). We recently observed that BNP inhibited cell proliferation and the MAP kinase activity induced by angiotensin II to the same degree as ANP using cultured rat mesangial cells (T. Suganami et al., unpublished observations). It is conceivable, therefore, that these mechanisms in combination could have worked under the chronic excess of BNP in this renal ablation model. Nevertheless, additional studies obviously are needed to clarify the mechanisms involved. Studies are ongoing in our laboratory to explore the interaction between natriuretic peptides and the angiotensin-TGF-β axis in vitro and in vivo with other nephropathy models.

Although BNP prevented glomerular histologic changes in the present study, the effect of BNP on renal function is still inconclusive. Because the remnant kidneys tended to be greater in non-Tg than in BNP-Tg (Table 2), it seems unlikely that BNP-Tg had erroneously more renal mass left intact as a result of technical variations. BNP-Tg showed lower serum creatinine and less overt albuminuria (Figure 5). However, we should be cautious to interpret these data. First, there was no significant difference in creatinine clearances between the groups with renal mass reduction (Table 2). Although final body weights were not significantly different between them (Table 2), non-Tg tended to be slightly greater. Therefore, we cannot exclude the possibility that the food intake might have differed between the groups, giving the difference in serum creatinine. Also inconclusive is the influence of a possible...
difference in water intake, although the urine volume was not statistically different between the groups throughout the course. Furthermore, it should be noted that when compared with rat models of subtotal nephrectomy (17–19,37), mice that were subjected to this insult seemed relatively more resistant in terms of histologic and functional deterioration and therefore were not suitable for studying progressive renal failure; the reason for this species-specific difference is unclear. One recent study of this model in mice reported similar results to ours with mild functional and histologic changes up to 44 wk after nephrectomy (48). Therefore, whether BNP exerts beneficial effects on renal function requires further clarification with different models.

Whether the sustained reduction of systemic BP caused by excess of circulating BNP contributed to the effects observed is another issue to be addressed. To answer this question, we used chronic hydralazine administration. As shown in Figure 6, hydralazine treatment seemed to achieve effective BP reduction but failed to inhibit glomerular hypertrophy. These results suggest that the systemic hypotension may not be critically contributing to the effects exerted by BNP. Lafayette et al. (37) showed that despite BP reduction comparable to angiotensin converting enzyme inhibition or its receptor blockade, a combination therapy with reserpine, hydralazine, and hydrochlorothiazide did not exert beneficial effects on the kidney in rats subjected to renal ablation and attributed the failure to its inability to reduce glomerular transcapillary pressure in the latter regimen. By contrast, Griffin et al. (49) claimed that the efficacy of antihypertensive regimens on glomerular histology is directly proportional to their efficacy to achieve sustained BP reduction regardless of the regimen used and that the observed inferiority of the triple therapy may be secondary to its lability of BP profile. Because close monitoring of BP was not performed in the present study, we cannot rule out the possibility that the daily profile of BP changes might have been more labile in the hydralazine-treated group than in the BNP-Tg group, leading to less effect.

The natriuretic peptide system consists of three endogenous ligands that in turn stimulate two biologically active receptors, GC-A and GC-B (1,2,26). We already reported that BNP-Tg with higher copy numbers of the transgene showed marked skeletal overgrowth (28), with the excess of BNP probably activating the physiologic CNP/GC-B pathway in the bone to stimulate endochondral ossification. BNP-Tg line 55 used in

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*Values are expressed as mean ± SEM. Urine volume and urinary creatinine levels were measured as outlined in Materials and Methods. Values in parentheses in urine volume and urinary creatinine excretion indicate relative levels when the baseline level at week 0 is 1.0 in each group. n = 5–8.

b P < 0.05 compared with week 0.

c P < 0.01 compared with week 0.

d P < 0.05 compared with non-Tg.
the present study revealed mild skeletal phenotypes (28). It is important to clarify, therefore, whether the effects of BNP observed in this study are BNP specific or via mechanisms shared with other natriuretic peptides. In the kidney, BNP and ANP are equally potent stimuli for activation of GC-A, which is distributed widely in the mesangium, capillary, and tubules, whereas CNP selectively activates GC-B, whose expression is detected mainly in the tubular system (43). Kishimoto et al. (50), using mice that were deficient in GC-A, demonstrated that natriuretic/diuretic factors released from the heart in response to volume expansion act exclusively through GC-A in the mouse kidney. Together with the profound effects on glomerular histology observed in the present study, these findings suggest that the effects of BNP were mediated via GC-A.

Analyses of crosses between BNP-Tg and GC-A-null mice (30) and other combinations would give answers to these questions.

The data obtained so far suggest a possibility that natriuretic peptides might be therapeutically applicable in renal diseases. Administration of ANP has been used in both experimental and clinical settings (43) and reported to exert beneficial effects in various models of acute renal injury, such as ischemic acute renal failure (23,24,43). Whether the chronic administration of natriuretic peptides would exert beneficial effects on renal adaptation encountered after subtotal renal ablation may be caused in part by increased secretion of ANP, which may facilitate glomerular hyperfiltration (20–22). It cannot be denied, therefore, that the augmentation of natriuretic peptides is an important compensatory mechanism in an early-phase response to major nephron reduction. In a chronic phase, however, it is possible to surmise that the augmented natriuretic peptides, if any, may work to lessen further deterioration of glomerular histologic changes.

In summary, these data indicate that the chronic excess of BNP in mice ameliorates glomerular hypertrophy and mesangial expansion after renal ablation. The results also suggest that the observed effects of natriuretic peptides under reduced renal mass are not due merely to systemic BP reduction and therefore might be therapeutically applicable in various renal diseases. Although additional studies are needed to verify this current hypothesis, our findings could open up the possibility of a novel therapeutic strategy for chronic renal diseases, as well as an innovative application of natriuretic peptides to disorders other than congestive heart failure (12,13) and hypertension.

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