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Role of endothelin ET_B receptor in partial ablation-induced chronic renal failure in rats

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Abstract

We investigated the role of endothelin ET_B receptor in the remnant kidney model of chronic renal failure, by using the spotting-lethal (*sl*) rat, which carries a naturally occurring deletion in the endothelin ET_B receptor gene. After 5/6 nephrectomy, systolic blood pressure and renal functional parameters were measured for 12 weeks. At the end of the experimental period, arterial blood sample, remnant kidney, heart and aorta were collected and used for biochemical measurements and histopathological studies. The ET_B-deficient *sl*/*sl* rats exhibited earlier and higher increases in systolic blood pressure, urinary protein excretion, blood urea nitrogen and plasma creatinine concentration, compared with cases in wild-type rats. Histopathologic examination of the kidney revealed glomerular and tubular lesions, alterations of which were more severe in *sl*/*sl* than in wild-type rats. While aortic endothelin-1 contents were increased similarly in both groups, the level of renal endothelin-1 content was significantly elevated in *sl*/*sl* rats, but not in the wild-type rats. These results suggest that enhanced endothelin-1 production is at least partly responsible for the increased susceptibility to partial ablation-induced chronic renal failure in ET_B receptor-deficient rats and that ET_B receptor-mediated actions are protective against vascular and renal injuries in this disease.

Keywords: Endothelin-1; ET_B receptor; Chronic renal failure; Renal mass reduction; Hypertension

1. Introduction

Chronic renal failure is characterized by progressive loss of nephrons caused by increased intraglomerular pressure and hyperfiltration. The loss of autoregulatory ability exposes the glomeruli to the systemic blood pressure leading to glomerular hypertrophy and sclerosis (Brenner et al., 1982). The control of systemic and intraglomerular pressures attenuates the progression of renal insufficiency in both human and experimental animal models of chronic renal failure. Thus, the hyperfiltration should be considered as being a homeostatic disturbance and not a maladaptive response (Herrere-Acosta, 1994).

Angiotensin II is thought to be one of crucial factors in the progressive renal failure, since the peptide not only contributes to glomerular capillary hypertension but also directly stimulates the production of mesangial matrix proteins (Kagami et al., 1994). It has been reported that an angiotensin I converting enzyme inhibitor or an angiotensin II type 1 receptor antagonist exhibits a renoprotective effect in patients with chronic renal failure and in subtotally nephrectomized rats, the most frequently employed animal model (Maschio et al., 1996; Lewis et al., 1993; Anderson et al., 1986; Pollock et al., 1993).

On the other hand, it has been indicated that endothelin-1, an endothelium-derived potent vasoconstrictor peptide, is also involved in the pathogenesis of progressive renal failure of subtotally nephrectomized rats. Benigni et al. (1991) originally found that endothelin-1 production was enhanced in renal cortical tissues from rats with renal mass reduction. Orisio et al. (1993) noted that the progression of renal disease after renal mass reduction is closely related to an increase in renal endothelin-1 gene expression together with an excessive urinary excretion of the corresponding

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protein. Moreover, in rats with reduction of renal mass, chronic treatment with endothelin ET_A receptor antagonist reduced the urinary protein excretion, limited glomerular injury and prevented renal dysfunction (Benigni et al., 1993; Brochu et al., 1999). In hemodialysis patients with uremia, elevated plasma endothelin-1 levels have been reported, which correlated with the increase in blood pressure (Lebel et al., 1994). Taken together, it is reasonable to consider that endothelin-1 action mediated by ET_A receptors is at least partly contributive to the progression of partial ablation-induced chronic renal failure in rats and that a selective ET_A receptor antagonist may be a useful compound in the treatment of human progressive nephropathies.

Physiological and pathophysiological responses to endothelin-1 in various tissues are mediated by interactions with endothelin ET_A and ET_B receptor subtypes. Both subtypes on vascular smooth muscle cells mediate vasoconstriction, whereas the ET_B receptor subtype on endothelial cells mediates vasodilation possibly through the release of nitric oxide (NO) (Goto et al., 1996). As mentioned above, selective ET_A receptor antagonists were known to blunt the rise of blood pressure and to attenuate the development of glomerulosclerosis and vascular hypertrophy in chronic renal failure, at least in experimental animals (Benigni et al., 1993; Brochu et al., 1999). In addition, treatment with nonselective ET_A/ET_B receptor antagonists has also prevented the development of glomerular injury in the same uremic rats (Nabokov et al., 1996, 1999). On the other hand, Shimizu et al. (1999) demonstrated that the beneficial effects of an ET_A receptor antagonist on proteinuria and renal dysfunction in partial ablation-induced chronic renal failure rats were reversed by concomitant administration with an ET_B receptor antagonist. Thus, there is general agreement that ET_A receptor-mediated action plays a crucial role in the development of the ablation-induced chronic renal failure, although some conflicting finding about the effectiveness of an ET_A receptor antagonist has been observed (Pollock and Polakowski, 1997). On the other hand, the pathological role of ET_B receptor-mediated action in this disease model is not fully eluciated. The purpose of the present study was to examine the effect of loss of ET_B receptor-mediated action in the ablation-induced chronic renal failure in rats. We used the spotting-lethal (*sl*) rat, which carries a naturally occurring deletion in the ET_B receptor gene (Gariepy et al., 1996). Since homozygous (*sl*/*sl*) rats do not live beyond 1 month because of intestinal aganglionosis and resulting intestinal obstruction, dopamine β-hydroxylase promoter was used to direct ET_B transgene expression in *sl*/*sl* rats to support normal enteric nervous system development (Gariepy et al., 1998). These transgenic *sl*/*sl* rats live into adulthood and are healthy, expressing ET_B receptors in adrenal glands and other adrenergic neurons. They are ET_B-deficient in other tissues, but most important is the deficiency in the kidney, vascular endothelium, and vascular smooth muscle (Gariepy et al., 2000). Thus, the "rescued" ET_B receptor-deficient rats are a useful tool in determining the pathophysiological roles of ET_B receptors in renal and vascular tissues.

2. Materials and methods

2.1. Animals and experimental design

Two series of experiments were carried out. In the first series to verify the involvement of endothelin-1 in partial ablation-induced chronic renal failure, male 8-week-old Sprague–Dawley rats (Japan SLC, Hamamatsu, Japan) were used. In the second series, the "rescued" ET_B receptor-deficient rats (male) were used. The creation of transgenic *sl*/*sl* rats has been described previously (Gariepy et al., 1998). Homozygous (*sl*/*sl*) rats have dark eyes and pigmented coats only in small spots on their heads. Wild-type rats have pigmented heads, backs, and tails. To definitively differentiate these rats, polymerase chain reaction was performed on DNA isolated from tail biopsy specimens, as described (Gariepy et al., 1998). These *sl*/*sl* and wild-type rats, all of which were dopamine β-hydroxylase-ET_B transgenic, were used at 8 weeks of age.

All animals were allowed free access to standard laboratory rat chow and tap water and were housed under controlled humidity, temperature and a 12-h light/dark cycle. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

The remnant kidney model was induced by surgical renal reduction (5/6 nephrectomy) in two stages. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for all surgical procedures. Initially, a left midflank incision was made and the left kidney was exteriorized. The renal vessel was temporarily occluded with a hemostatic clamp and both poles of the kidney (two-thirds of the functioning kidney mass) were excised with scissors. Bleeding was controlled with thrombin (Mitsubishi Pharma, Tokyo, Japan) administered onto the cut surface. The kidney stump was returned to the abdominal cavity and the incision was closed. Two weeks later for recovery, the right kidney was exposed, the renal vessel and ureter were ligated with a silk suture, and the total kidney was removed. As the nonablated control, both stages of the sham operation with manipulation of the renal pedicles involved exteriorizing the kidney and subsequently replacing the intact kidney back into the abdominal cavity (sham-operated control).

After 5/6 nephrectomy, systolic blood pressure was monitored once a week by tail-cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). Overnight urine samples were collected from individual rats in metabolic cages for 12 weeks every 4 weeks. Blood samples were obtained from the tail vein at the end of each urine collection period. These samples were used for measurements of renal functional parameters. After the last urine

collection, all rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), exsanguinated, and the remnant kidney, heart, and aorta were collected and weighted. The thoracic aorta and renal tissue were used for morphometric analysis and endothelin-1 measurement.

2.2. Analytical procedure

Blood urea nitrogen, urinary protein and creatinine levels in plasma or urine were determined with the BUN-Test-Wako, Total Protein-Test-Wako and Creatinine-Test-Wako (Wako), respectively.

2.3. Histological studies

The remnant kidney and thoracic aorta of each rat were preserved in phosphate-buffered 10% formalin, after which the tissues were chopped into small pieces, embedded in paraffin, cut at 4 μm. Sections were stained with periodic acid-Schiff (kidney) or hematoxylin–eosin (aorta). To evaluate differences of glomerular size between wild-type and *sl*/*sl* rats, the diameters (μm) of 200–300 randomly selected glomeruli in each rat were measured using an objective micrometer (U-OCM, Olympus Optical, Tokyo, Japan), based on a method described by Otsuka et al. (1998).

Four different cross-sections of each vessel placed under microscope were photographed, and vessel wall area, wall thickness, wall-to-lumen ration were determined with an image analyzer (AE-6905C, ATTO, Tokyo, Japan). The cross-sectional area (wall area: S) of the vessels was calculated as: $S=\pi M(\mathrm{ED}-M)$, where M is wall (media) thickness and ED is the external diameter. ED was calculated as: $\mathrm{ED}=\mathrm{Le}/\pi$. M was calculated as: $M=(\mathrm{Le}-\mathrm{Li})/2\pi$. Le and Li are the total lengths of the adventitia and internal elastic membrane, respectively.

2.4. Endothelin-1 measurement

Endothelin-1 was extracted from the kidney and aorta as described previously (Fujita et al., 1995). Briefly, the kidneys and aortas were weighted and homogenized for 1 min in ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM *N*-ethylmaleimide). The homogenates were left overnight at 4 °C, then 0.4 volume of distilled water was added after which the homogenates were centrifuged at $1500\times g$ for 30 min and the resultant was stored. Aliquots of the supernatant were diluted 1:10 with a 0.09% trifluoroacetic acid solution and applied to Sep-Pak C_{18} cartridges. The sample was eluted with 3 ml of 63.3% acetonitrile and 0.1% trifluoroacetic acid in water. Eluates were dried in a centrifugal concentrator, and the dried residue was reconstituted in assay buffer for radioimmunoassay. The clear solution was subjected to radioimmunoassay. The recovery of endothelin-1 was approximately 80%. Radioimmunoassay for tissue endothelin-1 was done, as described elsewhere (Matsumura et al., 1990), using endothelin-1 antiserum (a generous gift from Dr. Marvin R. Brown, Department of Medicine, University of California, San Diego, CA). This serum dose not cross-react with big endothelin-1.

2.5. Statistical analysis

Values were expressed as mean ± S.E.M. For statistical analysis, we used the unpaired Student's *t*-test for two-group comparison and one-way analysis of variance (ANOVA) followed by Tukey's tests for multiple comparison. Renal functional parameters in each group were analysed by repeated measures using one-way ANOVA combined with Dunnett's multiple range test. Statistical analysis for the difference in time course of systolic blood pressure between wild-type and *sl*/*sl* rats was performed using the two-way repeated ANOVA. For all comparisons, differences were considered significant at $P<0.05$.

3. Results

3.1. Effects of renal mass reduction in Sprague–Dawley rats

First, to confirm the participation of endothelin-1 in chronic renal failure induced by renal mass reduction, systolic blood pressure, aortic and renal endothelin-1 contents, and renal functional parameters were measured in both sham-operated and 5/6 nephrectomized groups, using male Sprague–Dawley rats. As shown in Table 1, at 12 weeks after ablation, systolic blood pressure, aortic and renal endothelin-1 contents, plasma creatinine level urine flow, urinary excretion of protein were significantly increased in rats with reduced renal mass, compared with the sham-operated control. These observations strongly

Table 1
Comparative data on body weights, systolic blood pressure, renal and aortic endothelin-1 (ET-1) contents and renal functional parameters in sham-operated control and renal mass reduction (RMR) animals at the end of experimental period

Group	*n*	Body weight (g)	SBP (mm Hg)	Renal ET-1 content (ng/g tissue)	Aortic ET-1 content (ng/g tissue)
Sham	9	472 ± 10	118 ± 2	0.27 ± 0.02	0.79 ± 0.09
RMR	11	456 ± 8	154 ± 4[a]	0.65 ± 0.06[a]	1.05 ± 0.28[b]

Group	*n*	UF (μl/min/kg BW)	UproV (mg/24 h/kg BW)	Ccr (ml/min/kg BW)	Pcr (mg/dl)
Sham	9	17.3 ± 0.8	32.5 ± 3.3	4.95 ± 0.67	0.71 ± 0.07
RMR	11	64.6 ± 5.5[a]	301.9 ± 47.2[a]	2.37 ± 0.24[b]	1.15 ± 0.08[a]

Each value represents mean ± S.E.M. SBP, systolic blood pressure; UF, urine flow; Uprov, urinary excretion of protein; Ccr, creatinine clearance; Pcr, plasma creatinine; sham, sham-operated control; RMR, renal mass reduction.

[a] $P<0.01$, compared with sham rats.

[b] $P<0.05$, compared with sham rats.

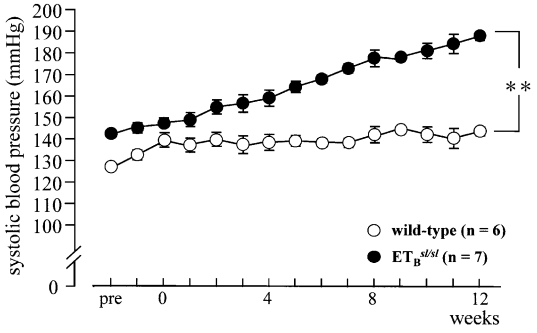

Fig. 1. Time course for systolic blood pressure in wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction. Values are means ± S.E.M. $^{**}P<0.01$, wild-type vs. $ET_B^{sl/sl}$.

support a role for endothelin-1 in the pathogenesis of hypertension and the progression of renal insufficiency in chronic renal failure, as reported by others (Benigni et al., 1991, 1993; Orisio et al., 1993; Brochu et al., 1999).

3.2. Changes in systolic blood pressure in ET_B-deficient *sl/sl* and wild-type rats

The systolic blood pressure of ET_B-deficient *sl/sl* and wild-type rats before the 5/6 nephrectomy was 142 ± 2 and 127 ± 1 mm Hg, respectively ($P<0.01$). As shown in Fig. 1, systolic blood pressure was progressively elevated after the 5/6 nephrectomy in both groups. However, the development of hypertension in the *sl/sl* group was much more marked than the case of the wild-type ($P<0.01$). At 12 weeks, the systolic blood pressure of the homozygous and wild-type animals was 188 ± 6 and 141 ± 2 mm Hg, respectively, being statistically significant ($P<0.01$) compared with each basal value before 5/6 nephrectomy. In sham-operated control animals, there were no significant increases in systolic blood pressure during 12-week period, in both groups, although the values at 0 and 12 weeks tended to be higher in the *sl/sl* animals (*sl/sl*: 135 ± 3 mm Hg at 0 week and 142 ± 6 mm Hg at 12 week; wild-type: 125 ± 3 mm Hg at 0 week and 126 ± 5 mm Hg at 12 week).

3.3. Changes in renal function in ET_B-deficient *sl/sl* and wild-type rats

Fig. 2 represents changes in renal functional parameters at 0, 4, 8 and 12 weeks after the 5/6 nephrectomy. Urinary excretion of protein in the *sl/sl* rats was progressively and markedly increased, compared with cases in wild-type rats. In contrast, there were no remarkable changes in wild-type rats, throughout the 12-week experimental period. Qualitatively similar results were observed in the changes of blood urea nitrogen. Plasma creatinine levels were transiently elevated immediately after the 5/6 nephrectomy (0 week) in both groups, thereafter the increased level returned to the basal level in the wild-type but not the *sl/sl* rats. These changes corresponded to those in creatinine clearance. In sham-operated controls, no significant alterations were observed in each variable during the 12-week period, in both groups (data not shown).

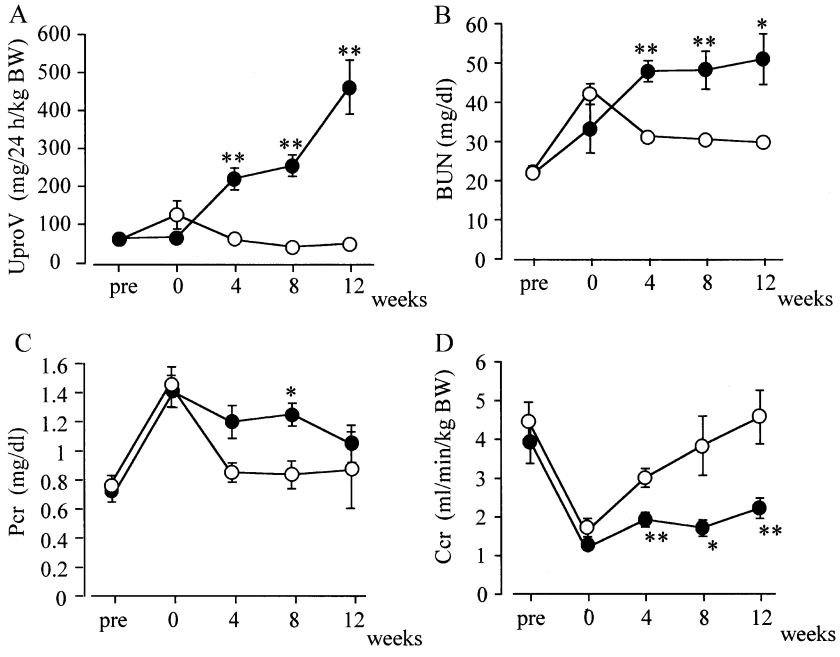

Fig. 2. Time course for urinary excretion of protein (UproV, A), blood urea nitrogen (BUN, B), plasma creatinine level (Pcr, C) and creatinine clearance (Ccr, D) in wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction. Values are means ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$ vs. wild-type at the corresponding time. wild-type (open circle, n = 6), $ET_B^{sl/sl}$ (closed circle, n = 7).

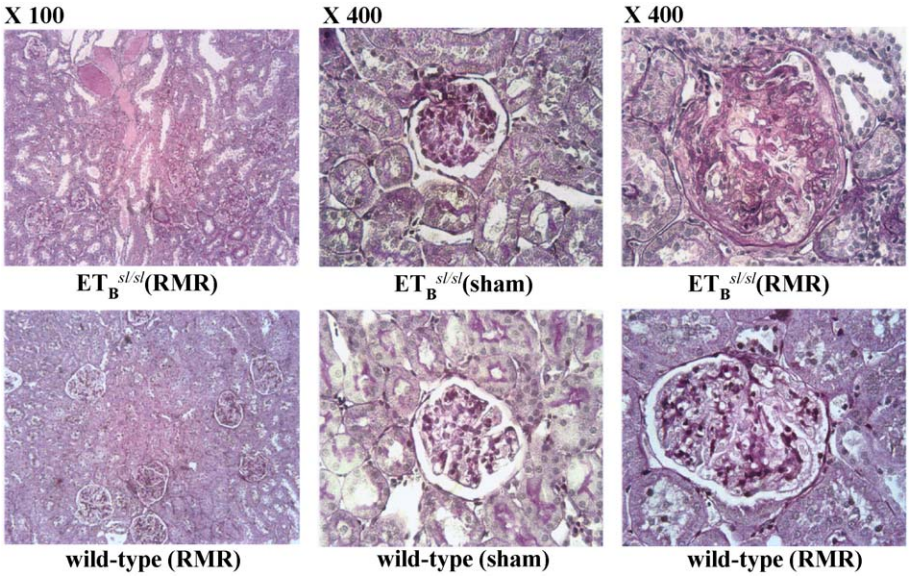

Fig. 3. Representative light micrographs of renal tissues obtained from wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction (RMR) or sham-operation (sham) (periodic acid-Schiff staining).

3.4. Renal histological findings in ET_B-deficient sl/sl and wild-type rats

Fig. 3 shows typical examples in renal tissues of the wild-type and *sl/sl* animals. Compared with the case of wild-type rat, histological examination of the kidney in *sl/sl* rats at 12 weeks after 5/6 nephrectomy revealed severe damage characterized by tubular dilatation and atrophy, proteinaceous cast in tubuli, interstitial cell infiltration, thickening of small arteries and fibrinoid-like necrosis in glomeruli (left, upper panel). Compared with each sham-operated animals, glomerulosclerotic lesions were observed in both renal mass reduction animals, but the degree was much more severe in the kidney of *sl/sl* rats (right panels). In addition, we

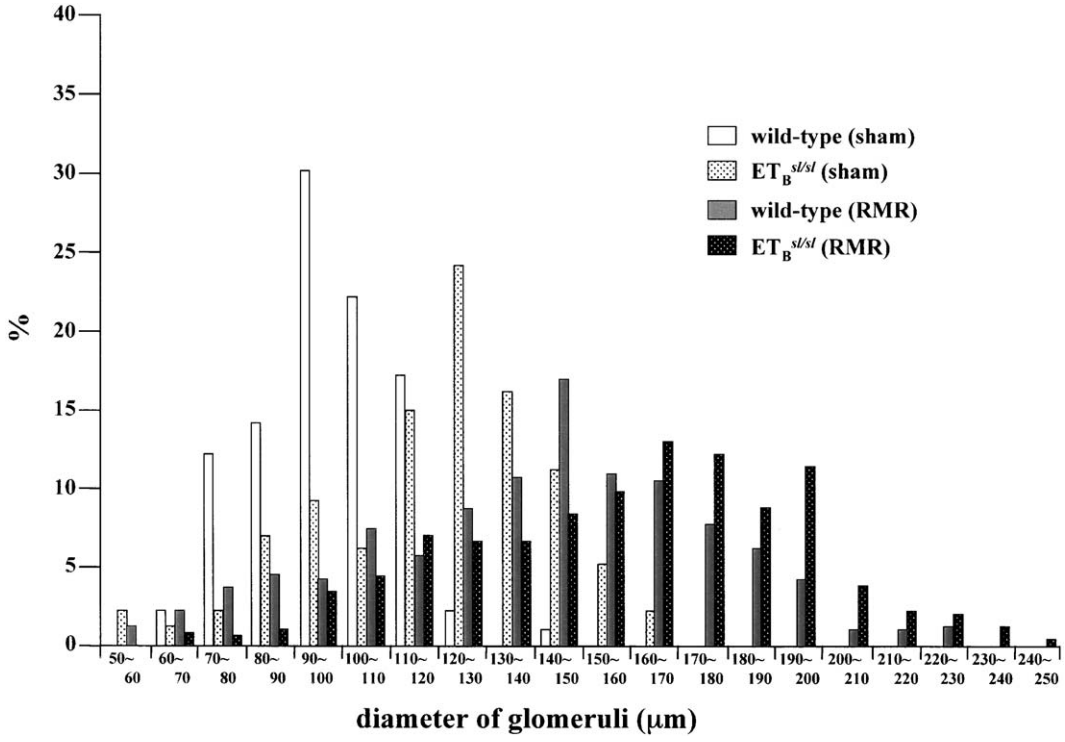

Fig. 4. Comparative data on glomerular diameter of wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction (RMR) or sham-operation (sham). Each column represent mean value of three rats.

examined the glomerular sizes in the four groups. As shown in Fig. 4, the glomerular size distribution indicated that the glomeruli of renal mass reduction animals were larger than those of each sham animals, and those of *sl/sl* animals tended to be larger compared with cases of wild-type animals.

3.5. Morphological analysis of aorta in ET_B-deficient sl/sl and wild-type rats

Fig. 5 shows typical examples of representative cross-sections of thoracic aorta obtained from one each of sham-operated control and 5/6 nephrectomized rats in both the *sl/sl* and wild-type groups, at 12 weeks. Increase in vascular medial thickness (wall thickness), a characteristic finding for hypertensive arterial hypertrophy, was evident in *sl/sl* 5/6 nephrectomized rats. The data on morphometric analysis are summarized in Table 2. ET_B-deficient *sl/sl* and wild-type 5/6 nephrectomized rats revealed significant increases in the wall thickness, wall area and wall-to-lumen ratio at 12 weeks when compared with each sham-operated group (except for wall-to-lumen ratio in the wild-type). The changes of these parameters of vascular hypertrophy were more evident in *sl/sl* than in wild-type 5/6 nephrectomized rats. In addition, there was a significant increase in heart weight of *sl/sl* 5/6 nephrectomized rats.

3.6. Renal and aortic endothelin-1 contents in ET_B-deficient sl/sl and wild-type rats

When renal endothelin-1 content was determined at 12 weeks, there was a significant increase in *sl/sl* 5/6 nephrectomized rats, compared with *sl/sl* sham-operated control rats, but this is not the case in the wild-type group. In contrast, aortic endothelin-1 content was increased significantly in both *sl/sl* and wild-type 5/6 nephrectomized rats, to the same extent (Fig. 6).

Table 2
Morphological analysis of aortas in wild-type and $ET_B^{sl/sl}$ rats at the end of the experimental period

Group	*n*	Heart weight (g/kg BW)	Wall thickness (μm)	Wall area (mm^2)	Wall-to-lumen ratio
Wild-type					
Sham	6	2.61 ± 0.033	95 ± 4	0.478 ± 0.024	0.271 ± 0.012
RMR	6	2.43 ± 0.049	113 ± 1[a]	0.663 ± 0.006[a]	0.276 ± 0.004
$ET_B^{sl/sl}$					
Sham	5	2.57 ± 0.036	109 ± 4	0.52 ± 0.027	0.337 ± 0.017
RMR	7	2.83 ± 0.015[c]	142 ± 8[b,c]	0.821 ± 0.053[b,c]	0.368 ± 0.022[d]

Values are mean ± S.E.M. Sham, sham-operated control; RMR, renal mass reduction; BW, body weight.
[a] $P<0.01$ vs. wild-type sham rats.
[b] $P<0.01$ vs. $ET_B^{sl/sl}$ sham rats.
[c] $P<0.05$ vs. wild-type RMR rats.
[d] $P<0.01$ vs. wild-type RMR rats.

4. Discussion

Previous studies have demonstrated the close relationship between the renal endothelin-1 overproduction and the progression of renal injury in the partial ablation-induced chronic renal failure in rats (Benigni et al., 1991; Orisio et al., 1993). In the present study, we also observed the increment of endothelin-1 contents in renal and vascular tissues of the remnant kidney model, using male Sprague–Dawley rats. Chronic treatment with selective endothelin ET_A receptor antagonists or nonselective ET_A/ET_B receptor antagonists is known to attenuate efficiently the above diseases, thereby suggesting that endothelin ET_A receptor-mediated endothelin-1 action at least partly plays a crucial role in the development of the ablation-induced chronic

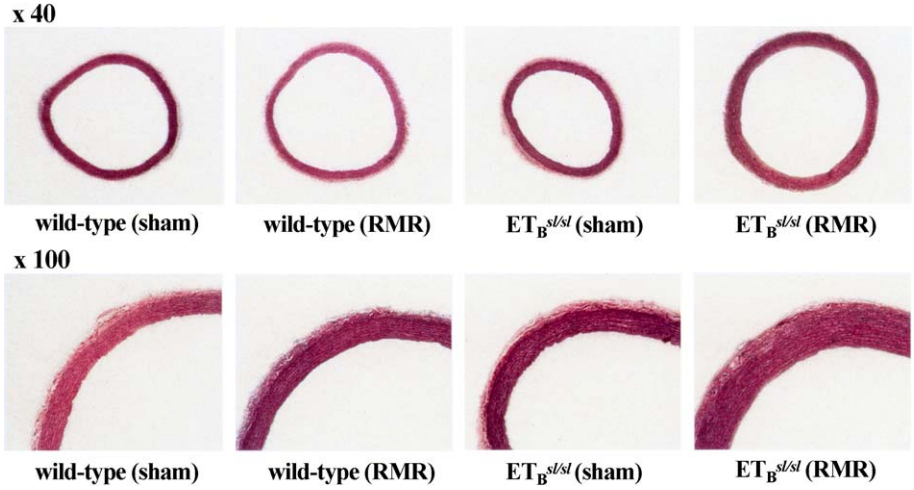

Fig. 5. Representative light micrographs showing cross-sections of thoracic aortas obtained from wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction (RMR) or sham-operation (sham) (hematoxylin–eosin staining).

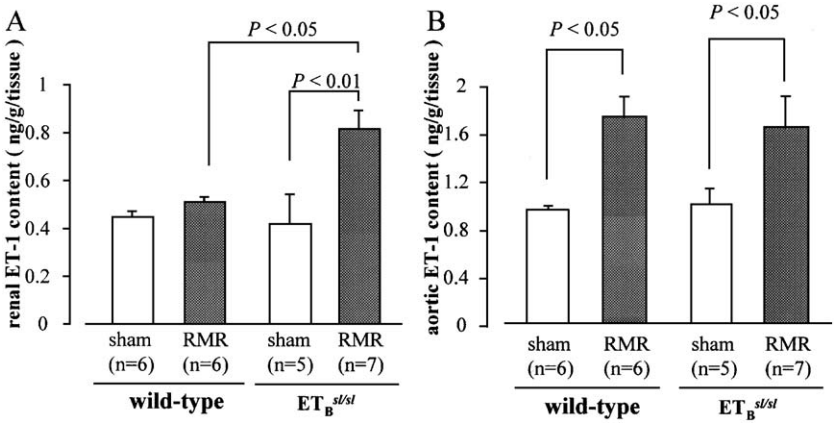

Fig. 6. Comparative data on renal (A) and aortic (B) endothelin-1 (ET-1) contents of wild-type and ET_B receptor-deficient *sl/sl* ($ET_B^{sl/sl}$) rats with renal mass reduction (RMR) or sham-operation (sham). Values are means ± S.E.M.

renal failure (Benigni et al., 1993; Brochu et al., 1999; Nabokov et al., 1996, 1999). However, the pathological role of endothelin ET_B receptor-mediated action in this disease is not fully elucidated. This led us to evaluate the renal and vascular responses to the partial ablation in the "rescued" ET_B receptor-deficient *sl/sl* rats, the animals which are useful in examining the pathophysiological roles of endothelin ET_B receptors in renal and vascular tissues (Matsumura et al., 2000). Results clearly indicated that the *sl/sl* rats revealed higher and earlier increases in blood pressure and progression of renal functional insufficiency. In addition, there were severe glomerular and tubular lesions, enlargement of glomeruli, notable cardiovascular hypertrophy, and increments of renal and aortic endothelin-1 contents in the *sl/sl* rats, compared with cases seen in wild-type animals. Thus, our findings suggest that endothelin ET_B receptor-mediated events in renal tissues and vasculature are protective to the development of the ablation-induced progressive renal failure. This view is consistent with the pharmacological evidence by Shimizu et al. (1999), who observed that chronic blockade of the ET_B receptor with a selective ET_B receptor antagonist reversed the beneficial effect of an ET_A receptor antagonist on renal functional impairment in the remnant kidney model.

There is a general agreement that endothelin-1 peptide level is elevated in renal tissues of rats with reduced renal mass, particularly in glomeruli and blood vessels. On the other hand, there are conflicting data regarding plasma endothelin-1 concentrations in the same animal model (Benigni et al., 1991; Brochu et al., 1999; Larivière et al., 1998), thereby supporting the view that locally produced endothelin-1, rather than circulating endothelin-1, is responsible for the ablation-induced progressive renal failure. Using *sl/sl* rats with reduced renal mass, we also observed that endothelin-1 level in whole renal tissue was significantly increased, although the circulating peptide concentration was not determined. Based on that renal endothelin-1 gene expression is increased in the remnant kidney after renal mass reduction (Orisio et al., 1993), the above elevation of renal endothelin-1 peptide level seems to reflect the enhancement of endothelin-1 biosynthesis. However, since clearance and/or metabolism of the peptide may be altered in this disease model, as suggested by Brochu et al. (1999), further studies to evaluate the endothelin-1 biosynthesis in the remnant kidney are required.

It is reasonable to consider that the severe damage observed in the ET_B receptor-deficient *sl/sl* rats is due to the loss of ET_B receptor-mediated renal and vascular functions. On the other hand, selective ET_A receptor antagonists and nonselective ET_A/ET_B receptor antagonists similarly improved the partial ablation-induced chronic renal failure (Nabokov et al., 1996). These results suggest that the antagonism of ET_A receptor is essential for the protection from the progressive renal injury, irrespective of the presence of ET_B receptor. Recently, we have noted that exaggerated renal and vascular injuries in the ET_B deficient *sl/sl* rats with mineralocorticoid-dependent hypertension were markedly ameliorated by the chronic treatment with selective ET_A receptor antagonist (Matsumura et al., 2000), indicating an important role of ET_A receptor-mediated event under the ET_B receptor deficiency. We also observed that ET_B receptor-deficient rats exhibit increased sensitivity to ET_A-mediated hypertensive and vasoconstrictor actions induced by exogenous endothelin-1, both in vivo and in vitro. Thus, it remains to be determined whether the loss of ET_B receptor by itself or the activation of ET_A receptor-mediated action secondary to the ET_B deficiency is contributive to the severe damage in response to the renal mass reduction in the ET_B deficient *sl/sl* rats.

In our study, the development of hypertension, renal dysfunction, glomerular and tubular lesions, and cardiovascular hypertrophy induced by the 5/6 nephrectory of the wild-type animals was considerably mild, compared with those in Sprague–Dawley rats. This may be explained by the fact that the wild-type rats utilized in our study are dopamine β-hydroxylase-ET_B transgenic (Gariepy et al., 1998). It has been reported that catecholamine secretion in the adrenal gland and norepinephrine overflow in response

to the renal nerve stimulation are suppressed by the activation of ET_B receptors (Matsuo et al., 1997; Nagayama et al., 2000). In addition, renal structural and functional damage in subtotally nephrectomized rats are known to be attenuated by moxonidine, at subantihypertensive doses, an agent which reduces the efferent sympathetic nerve activity (Amann et al., 2000), suggesting that circulating catecholamines and/or renal sympathetic nerve activity is involved in the pathogenesis of the ablation-induced progressive renal failure. Thus, dopamine β-hydroxylase-ET_B transgene in adrenal glands and other adrenergic neurons may be responsible for the lower sensitivity to the partial ablation-induced injury. Similar findings have been observed in this wild-type rats with deoxycorticosterone acetate-salt-induced hypertension (Matsumura et al., 2000), the pathogenesis of which is closely related to the sympathetic nervous system (Garvas and Garvas, 1989). Alternatively, the lower sensitivity of wild-type rats may be related to differences in the rat strains. To clarify this problem, further studies using non-transgenic wild-type rats are needed.

The rat remnant kidney model has been extensively used to investigate the mechanisms responsible for the progressive nature of human renal disease. This model is characterized by the development of hypertensive proteinuria and progressive glomerulosclerosis (Klahr et al., 1988; Bidani et al., 1987). Mesangial cell proliferation, which is widely accepted as an action mediated through the endothelin ET_A receptor (Ohlstein et al., 1992; Kohno et al., 1994), is considered to be a major mechanism involved in the glomerulosclerosis (Fukuda et al., 1996). Although the pathophysiological roles of endothelin ET_B receptor in the kidney are not fully understood, several studies have shown that the activation of this subtype produces a endothelium-dependent renal vasodilator effect through the release of NO and prostaglandin I_2 (De Nucci et al., 1988; Takayanagi et al., 1991), and a natriuretic effect probably via the inhibition of sodium reabsorption at the tubular level (Brooks et al., 1994; Hashimoto et al., 1998). We have recently noted that the endothelin ET_B receptor-mediated natriuresis is also due to the enhanced generation of NO (Konishi et al., 2002). In addition, NO is known to inhibit the mitogenesis and the proliferation of vascular smooth muscle cells (Garg and Hassid, 1989). A supplementation of L-arginine, a NO precursor, can antagonize endothelin-1-induced mesangial cell proliferation (Mattana and Singhal, 1995) and ameliorates the progression of renal disease in rats with subtotal nephrectomy (Reyes et al., 1992), whereas chronic treatment with NO synthase inhibitor aggravates glomerular injury in the same animal model (Fujihara et al., 1995). Taken together, endothelin ET_B receptor–NO pathway may function as an antiproliferative factor of mesangial cells against the progressive glomerulosclerosis. Therefore, the loss of ET_B receptor in the glomerulus, which would occur in the mesangial cells, may exert a deleterious effect and further exacerbate ET_A receptor-mediated mesangial proliferation, leading to the development and maintenance of glomerulosclerosis in the remnant kidney. However, further studies are still required in order to clarify the contribution of NO in the ET_B receptor-mediated event in the remnant kidney.

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