Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 468 (2003) 209–216
www.elsevier.com/locate/ejphar

Nephroprotective effects of the endothelin ET_A receptor antagonist darusentan in salt-sensitive genetic hypertension

Lars Rothermund[a,b], Tobias Traupe[a], Maike Dieterich[a], Peter Kossmehl[a], Chana Yagil[c], Yoram Yagil[c], Reinhold Kreutz[a,b,*]

[a] *Institut für Klinische Pharmakologie und Toxikologie, Benjamin Franklin Hospital, Freie Universität Berlin, Berlin, Germany*
[b] *Medizinische Klinik IV Nephrologie, Benjamin Franklin Hospital, Freie Universität Berlin, Berlin, Germany*
[c] *Laboratory for Molecular Medicine and Department of Nephrology and Hypertension, Faculty of Health Sciences, Ben-Gurion University, Barzilai Medical Center Campus, Ashkelon, Israel*

Received 16 January 2003; received in revised form 21 March 2003; accepted 4 April 2003

Abstract

We tested the effect of selective endothelin ET_A receptor blockade on the development renal damage in the Sabra rat model of genetic salt-sensitivity. Animals from the salt-sensitive (SBH/y) and salt-resistant strains (SBN/y) were either salt-loaded with deoxycorticosterone acetate and salt (DOCA) or fed a normal diet. Additional salt-loaded groups were also treated with the selective ET_A antagonist darusentan (DA). Salt-loading in SBH/y increased systolic blood pressure by 75 mm Hg and urinary albumin excretion 23-fold ($P<0.0001$). Darusentan attenuated the rise of systolic blood pressure (50%) and urinary albumin excretion (63%, $P<0.01$, respectively). Salt-loading in SBH/y was associated with significant increased osteopontin mRNA expression as well as glomerulosclerosis and tubulointerstitial damage in the kidney ($P<0.05$, respectively). This was either significantly reduced or normalized by darusentan ($P<0.05$, respectively). Thus, darusentan confers a significant renal protection in the Sabra model of salt-sensitive hypertension.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Salt; Hypertension; Endothelin; Kidney, rat; Genetic

1. Introduction

It has recently been demonstrated that salt-sensitive hypertension develops in response to high salt-intake and treatment with deoxycorticosterone acetate (DOCA) in rats and mice that are deficient for endothelin ET_B receptors (Ohuchi et al., 2000). These data, as well as recent in vitro studies (Gilmore et al., 2001; Gallego and Ling, 1996), suggest that endothelin-1 is able through its action on ET_B receptors to maintain normal arterial pressure during salt-loading. This is possibly mediated by tonic inhibition of the epithelial sodium channel in the cortical collecting duct and subsequently by a decrease in sodium reabsorption. In contrast, activation of endothelin ET_A receptors may contribute to the development of salt-sensitive hypertension, allegedly by inducing sodium reabsorption through activation of the epithelial sodium channel (Ohuchi et al., 2000). Consequently, selective ET_A-blockade may be useful in the treatment of hypertension and in preventing target organ damage not only by inhibiting vasoconstriction and the proliferative properties of endothelin-1, but also through its effects on renal sodium handling (Schiffrin, 1999; Pollock, 2000).

The Sabra rat model of salt-susceptibility represents a powerful genetically derived animal model to study salt-sensitive hypertension and related target organ damage (Yagil et al., 1996). While the salt-sensitive SBH/y strain develops significant hypertension and target organ damage during salt-loading with DOCA-salt, the reference salt-resistant SBN/y strain maintains normal blood pressure during this treatment. In addition, during salt-loading SBH/y develops renal injury, whereas the kidney in SBN/y remains unaffected. In a recently published study we reported that endothelin ET_A receptor blockade has an antihypertensive effect in DOCA-salt treated SBH/y and prevents left ventricular hypertrophy and dysfunction (Rothermund et al., 2002). In the current study the same cohort was used to test the hypothesis that the selective endothelin ET_A receptor antagonists darusentan

* Corresponding author. Department of Clinical Pharmacology, Benjamin Franklin Klinikum, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin, Germany. Tel.: +49-30-8445-2280; fax: +49-30-8445-4482.
E-mail address: Kreutz@medizin.fu-berlin.de (R. Kreutz).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01714-X

(DA; Nakov et al., 2002) confers resistance to the development of target organ damage in the kidney during salt-loading in the salt-sensitive SBH/y strain.

2. Materials and methods

2.1. Animals and experimental design

Male SBH/y and SBN/y were obtained from our colony at the Barzilai Medical Center campus of the Ben-Gurion University in Israel (Yagil et al., 1996). Rats were randomly assigned to experimental groups at the age of 8 weeks. Animals were either sham operated and fed standard rat chow (SSNIFF, Soest, Germany) and provided tap water ad libitum (groups SBN/y and SBH/y, respectively) or salt-loaded by subcutaneous implantation of a 75-mg sustained release deoxycorticosterone-acetate (DOCA) pellet (Innovative Research, Tampa, FL, USA) and providing 1% NaCl as drinking water for 8 weeks (groups SBN/y-DOCA and SBH/y-DOCA) (Rothermund et al., 2002). Two additional groups of salt loaded animals were treated for 8 weeks orally with 50 mg kg^{-1} day^{-1} of the selective endothelin ET_A receptor antagonist darusentan (DA; SBN/y-DOCA-DA and SBH/y-DOCA-DA; Abbott, Ludwigshafen, Germany), which was mixed in standard rat chow (Rothermund et al., 2002).

2.2. Blood pressure measurement

Systolic blood pressure was measured in awake rats with a computer-controlled non-invasive blood pressure monitoring system applying the tail-cuff method (TSE, Bad Homburg, Germany). Systolic blood pressure was determined 8 weeks after initiation of the experimental protocol as reported (Kreutz et al., 2000; Rothermund et al., 2002).

2.3. Urine and biochemical analysis

Urine was collected in metabolic cages and assayed for sodium, albumin and endothelin-1 concentration, allowing calculation of 24 h urinary albumin excretion, sodium and endothelin-1 excretion. Urinary albumin excretion was determined by enzyme-linked immunosorbent assay (ELISA; Kreutz et al., 2000) using a rat specific antibody (ICN Biomedicals, Eschwege, Germany). Urinary sodium was determined by a standard technique. Urinary endothelin-1 concentration was measured using a commercially available ELISA for endothelin-1 (Immundiagnostik, Bensheim, Germany), suitable for direct measurement of endothelin-1 in the urine (Rothermund et al., 2001).

2.4. Organ preparation and analysis

Animals were sacrificed at the age of 16 weeks, while under pentobarbital anesthesia. Blood was drawn from the aorta. The kidneys were rapidly excised, rinsed in a 0.9% NaCl solution, blotted dry, and weighed. A mid-coronal section of the left kidney was immersed in Dubosq-Brasil solution and embedded in paraffin for histologic studies. The remaining tissues were immediately frozen in liquid nitrogen and stored at -80 °C until further analysis.

2.5. Morphological studies of the kidney

After embedding in paraffin, the kidneys were cut into 3 μm sections and stained with periodic acid Schiff (PAS), followed by hematoxylin counterstaining. Glomerular injury was assessed using the glomerulosclerosis index and tubulointerstitial changes, including tubular atrophy, dilation, casts, interstitial inflammation, and fibrosis, were assessed using the tubulointerstitial damage index with a semiquantitative scoring system as previously reported (Rothermund et al., 2001).

2.6. Osteopontin mRNA analysis

Osteopontin expression in the kidney was determined by Northern blot analysis. A 735 bp spanning cDNA fragment was generated by reverse transcription (RT)-PCR using the following primers: sense 5′ CTG TTC GGC CTT GCC TCC TGT CTC 3′ and antisense 5′ TGC ATC CGG CTT CTC GGC ACT ATC 3′ (based on the reported sequence GenBank accession number M14656) as previously reported. Northern blot analysis was performed as described (Kreutz et al., 1997).

2.7. Binding assays for endothelin ET_A and ET_B receptors

To analyze the renal expression of the two endothelin ET_A and ET_B receptor subtypes, binding assays were performed in the presence or absence of the subtype-specific endothelin receptor ligands (Rothermund et al., 2001).

2.8. Statistics

Data are expressed as mean ± S.E.M. Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Bonferroni's adjustment and by Mann–Whitney *U*-test. Analysis of correlation was performed using the Pearson's coefficient. Differences were considered significant at the level of $P<0.05$.

3. Results

3.1. Body and kidney weights

Body weight as well as relative kidney weights in relation to body weight at 16 weeks of age are presented in Table 1, respectively. Kidney weight of untreated SBN/y and SBH/y was similar. Salt-loading increased kidney

Table 1
Body and kidney weights

Experimental group	BW (g)	K BW^{-1} (mg g^{-1})
SBN/y	328 ± 5.4	2.4 ± 0.03
SBN/y-DOCA	319 ± 5.0	2.6 ± 0.05[a]
SBN/y-DOCA-DA	345 ± 6.9	2.7 ± 0.08
SBH/y	359 ± 3.1	2.6 ± 0.07[a]
SBH/y-DOCA	340 ± 8.9	3.0 ± 0.08
SBH/y-DOCA-DA	377 ± 7.4[a]	3.2 ± 0.06[b]

BW, body weight; K BW^{-1}, normalized kidney weight; SBN/y, salt-resistant Sabra rat strain; SBH/y, salt-sensitive Sabra rat strain; DOCA, deoxycorticosterone-acetate; DA, darusentan, selective endothelin ET_A receptor antagonist; $n = 8–15$ in each group.
[a] $P < 0.05$ vs. SBH/y-DOCA.
[b] $P < 0.001$ vs. SBH/y.

weight in both strains, and this effect was not prevented by endothelin ET_A receptor blockade.

3.2. Blood pressure

Systolic blood pressure is shown in Fig. 1 (panel A). Salt-loading for 8 weeks increased systolic blood pressure in SBH/y-DOCA by 75 mm Hg to a level that was significantly above that in SBN/y-DOCA ($P < 0.01$) or in untreated SBH/y ($P < 0.001$). Darusentan attenuated the blood pressure increase in response to salt-loading in the SBH/y-DOCA-DA group by 50% ($P < 0.001$), but systolic blood pressure was still elevated compared to untreated SBH/y ($P < 0.0001$).

3.3. Urinary albumin excretion

The data are shown in Fig. 1 (panel B). Urinary albumin excretion paralleled the blood pressure findings. Analysis of variance revealed a significant effect of strain, salt-loading and endothelin ET_A receptor blockade on urinary albumin excretion ($P < 0.005$). Salt-loading in SBH/y-DOCA increased urinary albumin excretion 23-fold compared to untreated SBH/y, and also compared to SBN/y-DOCA ($P < 0.0001$). Treatment with darusentan in SBH/y-DOCA-DA was associated with a 63% reduction in urinary albumin excretion compared to SBH/y-DOCA ($P < 0.05$), but urinary albumin excretion was still elevated compared to untreated SBH/y ($P < 0.01$).

3.4. Urinary sodium and endothelin-1 excretion

Urinary sodium and endothelin-1 excretion are presented in Fig. 2. DOCA treatment caused a significant and similar increase in urinary sodium excretion and endothelin-1 excretion ($P < 0.05$ and $P < 0.0001$, respectively) in both strains, which was not affected by ET_A blockade.

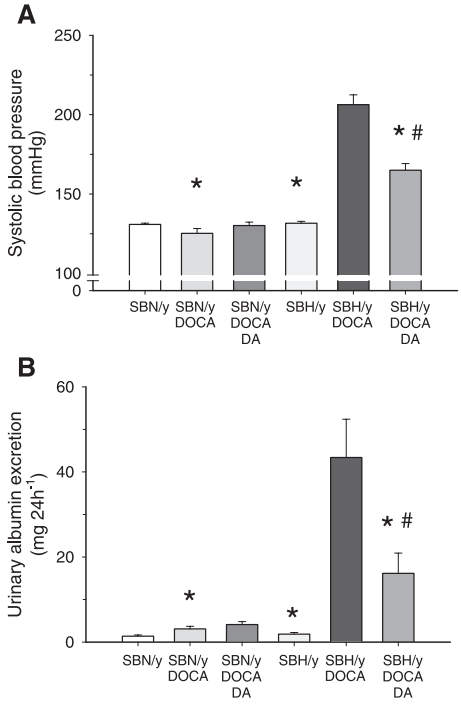

Fig. 1. (A) Systolic blood pressure; (B) Urinary albumin excretion; SBN/y, salt-resistant Sabra rat strain; SBH/y, salt-sensitive Sabra rat strain; DOCA, deoxycorticosterone-acetate; DA, darusentan, selective endothelin ET_A receptor antagonist; $n = 8–15$ in each group; *$P < 0.05$ vs. SBH/y DOCA. #$P < 0.05$ vs. SBH/y.

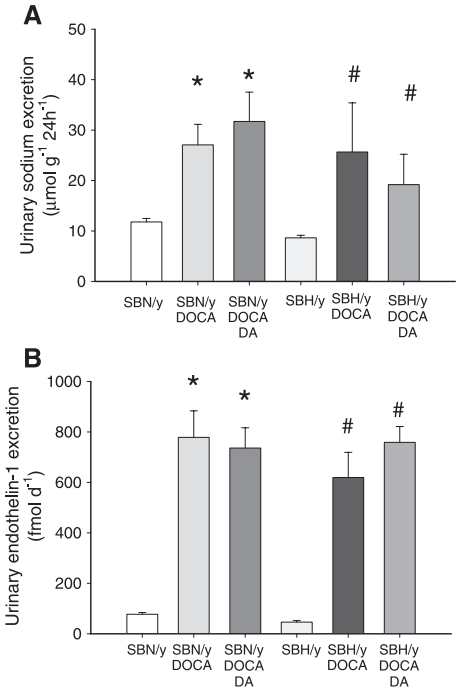

Fig. 2. (A) Urinary sodium excretion; (B) Urinary endothelin-1 excretion. SBN/y, salt-resistant Sabra rat strain; SBH/y, salt-sensitive Sabra rat strain; DOCA, deoxycorticosterone-acetate; DA, darusentan, selective endothelin ET_A receptor antagonist; $n = 8–15$ in each group; *$P < 0.05$ vs. SBN/y. #$P < 0.05$ vs. SBH/y.

3.5. Glomerulosclerosis and tubulointerstitial damage indices

The glomerulosclerosis index and tubulointerstitial damage index data are shown in Fig. 3 (panels A and B, respectively). Glomerulosclerosis index and tubulointerstitial damage index scores were similar in untreated SBN/y, SBH/y and salt-loaded SBN/y without and with treatment with darusentan (SBN/y-DOCA and SBN/y-DOCA-DA). After salt-loading, glomerulosclerosis index and tubulointerstitial damage index were significantly higher in SBH/y-DOCA compared to SBN-DOCA. ET-A blockade with darusentan reduced glomerulosclerosis index and tubulointerstitial damage index in SBH/y-DOCA-DA compared to SBH/y-DOCA ($P<0.05$), normalizing thereby to levels that were not different from SBN/y or SBH/y.

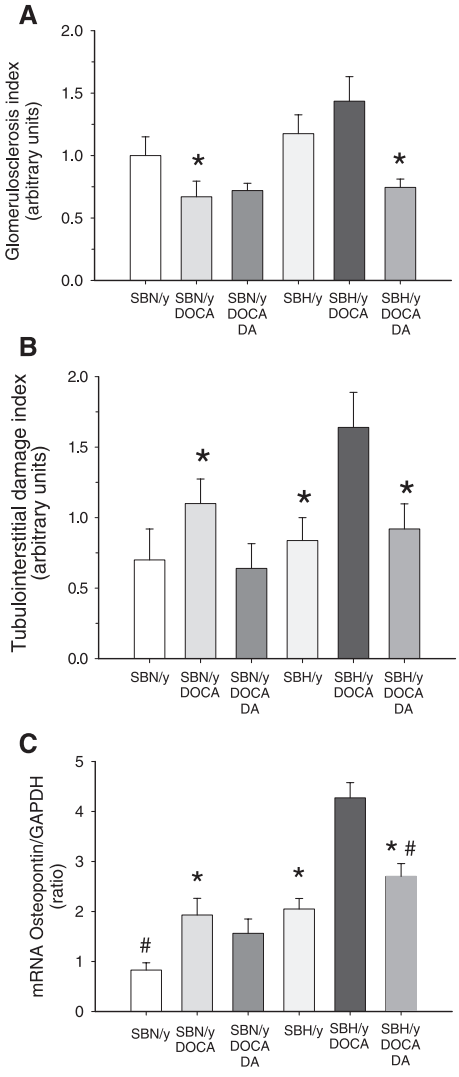

Fig. 3. (A) Glomerulosclerosis index; (B) Tubulointerstitial damage index; (C) Osteopontin mRNA expression. SBN/y, salt-resistant Sabra rat strain; SBH/y, salt sensitive-Sabra rat strain; DOCA, deoxycorticosterone-acetate; DA, darusentan, selective endothelin ET_A receptor antagonist; $n=8-15$ in each group; *$P<0.05$ vs. SBH/y DOCA. #$P<0.05$ vs. SBH/y.

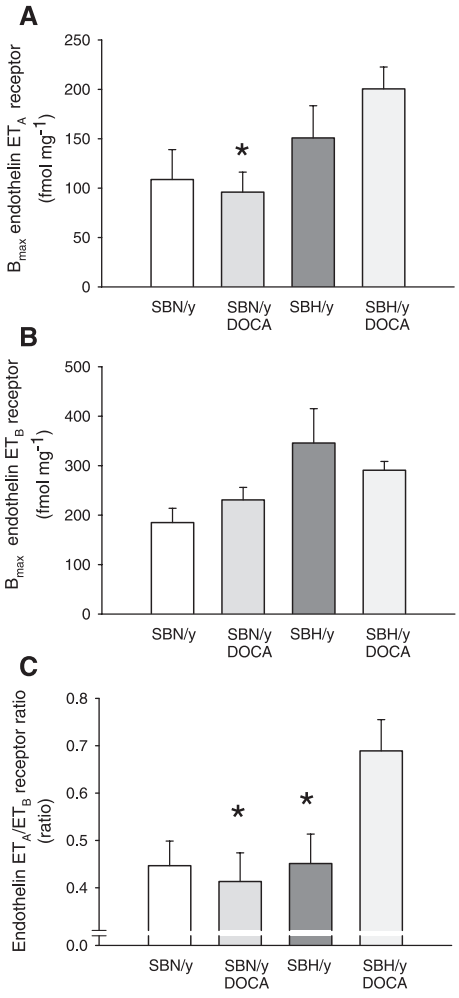

Fig. 4. (A) Endothelin ET_A receptor density; (B) Endothelin ET_B receptor densitiy; (C) Endothelin ET_A/ET_B receptor ratio. SBN/y, salt-resistant Sabra rat strain; SBH/y, salt-sensitive Sabra rat strain; DOCA, deoxycorticosterone-acetate; $n=6-7$ in each group; *$P<0.05$ vs. SBH/y DOCA.

3.6. Renal osteopontin mRNA expression

The data for renal osteopontin expression are shown in Fig. 3 (panel C). Renal osteopontin mRNA expression was higher in untreated SBH/y than in SBN/y ($P<0.01$). Salt-loading in SBN/y-DOCA and SBH/y-DOCA increased the expression further within each strain ($P<0.01$) and accentuated thereby the differences between the strains. Treatment with darusentan had no effect on osteopontin mRNA expression in SBN/y-DOCA-DA when compared to SBN/y-DOCA but was associated with a 37% reduction of this response in SBH/y-DOCA-DA compared to SBH/y-DOCA ($P<0.01$).

3.7. Endothelin ET_A and ET_B receptor binding in the kidney

Data for kidney endothelin receptor binding are presented in Fig. 4 and Table 2. Endothelin ET_A receptor density was significantly higher in SBH/y-DOCA compared

Table 2
Endothelin ET_A and ET_B receptor affinity (K_d) in the kidney

Experimental group	Endothelin ET_A receptor binding affinity [K_d] (nmol/l)	Endothelin ET_B receptor binding affinity [K_d] (nmol/l)
SBN/y	0.46 ± 0.03	0.61 ± 0.06[a]
SBN/y-DOCA	0.54 ± 0.12	0.77 ± 0.14
SBH/y	0.39 ± 0.06	0.32 ± 0.04
SBH/y-DOCA	0.53 ± 0.07	0.67 ± 0.06[a]

K_d, dissociation constant; SBN/y, salt-resistant Sabra rat strain; SBH/y, salt-sensitive Sabra rat strain; DOCA, deoxycorticosterone-acetate; $n = 6–7$ in each group.
[a] $P < 0.01$ vs. SBH/y.

to SBN/y-DOCA ($P < 0.05$), but no difference in endothelin ET_B receptor density was observed between these two groups. Endothelin ET_A/ET_B receptor ratio was significantly increased in SBH/y-DOCA compared to SBN/y-DOCA and in SBH/y-DOCA compared to untreated SBH/y ($P < 0.05$, respectively). No significant difference in endothelin ET_A receptor affinity was observed between the two strains or in response to DOCA (Table 2). Under normal dietary conditions, SBH/y showed a significantly higher endothelin ET_B receptor affinity compared to SBN/y ($P < 0.01$), while affinity was similar between SBH/y-DOCA and SBN/y-DOCA, due to a significant decrease in endothelin ET_B receptor affinity in the SBH/y strain in response to DOCA salt treatment ($P < 0.01$).

4. Discussion

The main aim of the present study was to test the hypothesis that pharmacological blockade of the endothelin ET_A receptor can attenuate the development of renal injury in the Sabra rat model of salt-sensitive genetic hypertension. This experimental model provides a powerful tool for the dissection between the effects of hypertension and salt-loading on target organ damage, since the SBN/y strain maintains normal blood pressure during salt-loading (Yagil et al., 1998; Crackower et al., 2002).

A case in point represents the current finding on urinary endothelin-1 excretion. In both groups that were salt-loaded (SBN/y-DOCA and SBH/y-DOCA), a significant increase in urinary endothelin-1 excretion occurred. This finding is in agreement with previous studies that have also demonstrated increased renal endothelin-1 content or urinary endothelin-1 excretion after salt-loading (Rothermund et al., 2001; Fujita et al., 1996). Interestingly, the data observed in SBN/y-DOCA animals clearly demonstrate that the increase in urinary endothelin-1 excretion is primarily due to salt-loading rather than to elevations in systolic blood pressure and urinary albumin excretion or renal damage. Thus, increased urinary endothelin-1 excretion per se does not appear to be associated with structural or functional renal changes. Therefore, salt-sensitivity of arterial hypertension and susceptibility to renal organ damage in the SBH/y strain must be attributable to mechanisms that are either independent or down-stream from renal endothelin-1 biosynthesis.

The increases of systolic blood pressure and renal target organ damage in SBH/y-DOCA are in keeping with recent studies, which report similar findings in other models of salt-sensitive hypertension (Kassab et al., 1998; Orth et al., 1998; Blezer et al., 1999; Matsumura et al., 1999; Schiffrin, 1999; Barton et al., 2000). In contrast to SBH/y-DOCA, no elevation in systolic blood pressure or any significant changes in glomerulosclerosis index, tubulointerstitial damage index, or urinary albumin excretion were observed in SBN/y-DOCA salt, indicating resistance towards the development of salt-sensitive hypertension and renal organ damage in the SBN/y strain.

As a parameter for renal inflammatory status we determined renal osteopontin mRNA expression. Osteopontin is a glycoprotein containing an adhesive arginine-glycine-aspartic acid sequence and acts as adhesion molecule as well as a macrophage chemotactic (Xie et al., 2001). Osteopontin has been shown to play a role in tubulointerstitial injury in several kidney disease models (Xie et al., 2001). After chronic angiotensin II infusion osteopontin upregulation has been demonstrated primarily in epithelial cells of the distal tubules, collecting duct and Bowman's capsule, which correlated with sites of monocyte/macrophage accumulation (Giachelli et al., 1994). We observed already a basal difference in renal osteopontin mRNA expression levels between the salt-resistant and the salt-sensitive strain. Osteopontin mRNA expression was 2.5-fold higher in SBH/y compared to SBN/y. Moreover, we found a significant increase in both strains after DOCA treatment. Overall, renal osteopontin expression appeared to parallel the extent of tubulointerstitial damage index (panel B and C in Fig. 3). This finding is in agreement with the observation of a significant correlation between renal osteopontin expression and blood pressure in a rat model of salt-sensitive hypertension in response to phenylephrine infusion (Johnson et al., 1999). Although the strong increase in renal osteopontin expression in response to DOCA salt in SBH/y animals was significantly diminished during treatment with darusentan, osteopontin mRNA levels were still elevated compared to SBH/y under normal diet. Our finding that osteopontin expression is already upregulated in SBH/y and parallels the extent of tubulointerstitial damage points to a link between osteopontin, tubulointerstitial damage index and the genetic susceptibility to develop salt-sensitive hypertension. A recently published study observed increased renal osteopontin expression in a model of renal injury induced by chronic hypokalemia (Suga et al., 2001) and chronic treatment of DOCA-salt in the Sabra rat is known to induce hypokalemia (Weinstock et al., 1984). Thus, hypokalemia could play a role in the induction of renal damage in the SBH/y-DOCA animals.

As reported previously, darusentan exhibited a considerable antihypertensive effect in salt-loaded SBH/y animals treated with this compound (Rothermund et al., 2002). This antihypertensive effect was paralleled by a similar decrease in urinary albumin excretion. The observed blood pressure-lowering and nephroprotective effect produced by endothelin ET_A receptor blockade is in agreement with other reports (Kassab et al., 1998; Orth et al., 1998; Blezer et al., 1999; Matsumura et al., 1999; Schiffrin, 1999; Barton et al., 2000, Pollock et al., 2000), but at variance with a short term study conducted for 3 weeks that showed only a temporary decrease of blood pressure in DOCA salt-loaded Sprague–Dawley rats after treatment with the endothelin ET_A receptor antagonist A-127722 (Pollock et al., 2000). This negative findings by Pollock et al. (2000) might be dependent on dose, time of initiation of treatment and possibly affinity of the endothelin ET_A receptor antagonist to the endothelin receptors. More interestingly, in rat models of nitric oxide synthase inhibition and two-kidney-one-clip hypertension endothelin ET_A receptor blockade exhibited only minor antihypertensive effects, although renal protection was present (Fujita et al., 1995; Verhagen et al., 1998). Therefore, it seems to be appropriate to distinguish between blood pressure independent nephroprotective effects and nephroprotection associated with antihypertensive effects of endothelin receptor antagonism in salt-sensitive forms of hypertension.

There is strong evidence that the blood pressure independent nephroprotective effects of endothelin receptor antagonism is mainly due to antinflammatory properties (for review, see Benigni et al., 2001). Proteins filtered in excessive amounts are reabsorbed by proximal tubular cells, leading to the upregulation of endothelin-1 in the interstitial compartment. Endothelin-1 binds to interstitial fibroblast receptors and interstitial fibroblasts are challenged by endothelin-1 proliferate and generate extracellular matrix. Moreover, endothelin-1 is chemotactic for blood monocytes and challenges them to secrete proinflammatory cytokines and growth factors, such as transforming growth factor beta, which contribute to interstitial remodeling (Benigni et al., 2001).

Nephroprotection associated with antihypertensive effects of endothelin receptor antagonism in salt-sensitive hypertension seem to be related to other pathomechanisms. In the DOCA-salt hypertensive rat model experimental evidence indicates that the endothelin ET_B receptor may serve to maintain lower arterial pressure (Pollock, 2000), a finding which was confirmed by data obtained from ETB receptor-deficient rats. In these endothelin ET_B receptor deficient animals a hypertensive phenotype developed only after salt-loading (Gariepy et al., 2000; Matsumura et al., 2000). This form of hypertension was completely ameliorated by amiloride, a highly selective inhibitor of the epithelial sodium channel in the distal nephron (Gariepy et al., 2000). The most likely explanation for these findings is derived from previous in vitro studies in distal nephron cells showing that endothelin-1 is capable of either inhibiting the epithelial sodium channel via the endothelin ET_B receptor or of stimulating the epithelial sodium channel via the endothelin ET_A receptor (Gallego and Ling, 1996).

Manifestation of salt-sensitive hypertension and target organ damage in SBH/y-DOCA is characterized by an increased renal endothelin ET_A/ET_B receptor ratio (Fig. 4, panel C). This finding is in agreement to a recently published study, in which the renal endothelin-system was stimulated in the salt-sensitive model of spontaneous hypertension of stroke-prone spontaneously hypertensive rats after uninephrectomy and salt-loading, but not in salt-resistant spontaneously hypertensive rats due to uninephrectomy and salt-loading (Rothermund et al., 2001). The pathophysiological relevance of renal endothelin ET_A/ET_B receptor disbalance may be further amplified by a reduced endothelin ET_B receptor affinity in SBH/y-DOCA salt compared with SBH/y rats, a finding that was also reported for SHRSP-NX-NaCl animals (Rothermund et al., 2001). The present study is the first to show that mineralocorticoid dependent hypertension is related to the status of endothelin ET_A/ET_B receptor balance. SBN/y-DOCA salt animals with a unchanged endothelin ET_A/ET_B receptor ratio are normotensive, without functional or structural kidney damage, whereas SBH/y-DOCA salt treated rats with an elevated endothelin ET_A/ET_B receptor ratio develop arterial hypertension and renal failure.

Taken together, we conclude that with an increased endothelin ET_A/ET_B receptor ratio rather than an increased urinary endothelin-1 excretion contributes to a higher susceptibility to development of DOCA-salt hypertension and kidney damage in SBH/y as compared to SBN/y strain. A possible mechanism is impairment of urinary sodium excretion via stimulation of the epithelial sodium channel. A genetic difference in the regulation of endothelin receptors appears to be possible. Our recent genetic linkage studies have identified several blood pressure quantitative loci in the rat genome that account for this phenotype in the SBH/y rat model (Yagil et al., 1998, 1999). In addition, based on chromosomal positional analysis the genes encoding ET_A and ET_B receptors and the genes involved in the biosynthesis of endothelins can be excluded as candidate genes for the genetic susceptibility to develop salt-sensitive hypertension in the SBH/y rat model. Moreover, a role of the alpha-, beta-, and gamma-subunit of the epithelial sodium channel are also unlikely to be responsible (Grunder et al., 1997). Thus, it appears of interest to determine as to how the endothelin system is related to the primary, i.e. genetic, susceptibility to develop salt-sensitivity in the SBH/y strain. The elucidation of the mechanisms involved and comparative genomic analysis between the rat and human genome (http://rgd.mcw.edu/) may provide new insights for the development of pharmacogenetic treatment strategies utilizing ET_A antagonists such as darusentan for the treatment of salt-sensitive hypertension and target-organ damage.

Although the genetic mechanisms responsible to confer hypertension and renal organ damage in the salt-sensitive Sabra rat strain are still unknown, the current study clearly demonstrates a pronounced renal protection against both functional, e.g. albuminuria, and structural renal tissue damage effect.

Acknowledgements

This study was supported by a grant to Dr. Kreutz by the Bundesministerium für Bildung und Forschung (Nationales Genomforschungsnetz, Herz-Kreislauf-Netz, Standort Berlin).

References

Barton, M., Vos, I., Shaw, S., Boer, P., D'Uscio, L.V., Grone, H.J., Rabelink, T.J., Lattmann, T., Moreau, P., Luscher, T.F., 2000. Dysfunctional renal nitric oxide synthase as a determinant of salt-sensitive hypertension: mechanisms of renal artery endothelial dysfunction and role of endothelin for vascular hypertrophy and glomerulosclerosis. J. Am. Soc. Nephrol. 11, 835–845.

Benigni, A., Perico, N., Remuzzi, G., 2001. Research on renal endothelin in proteinuric nephropathies dictates novel strategies to prevent progression. Curr. Opin. Nephrol. Hypertens. 10, 1–6.

Blezer, E.L., Nicolay, K., Goldschmeding, R., Jansen, G.H., Koomans, H.A., Rabelink, T.J., Joles, J.A., 1999. Early-onset but not late-onset endothelin-A-receptor blockade can modulate hypertension, cerebral edema, and proteinuria in stroke-prone hypertensive rats. Hypertension 33, 137–144.

Crackower, M.A., Sarao, R., Oudit, G.Y., Yagil, C., Kozieradzki, I., Scanga, S.E., Oliveira-dos-Santos, A.J., da Costa, J., Zhang, L., Pei, Y., Scholey, J., Ferrario, C.M., Manoukian, A.S., Chappell, M.C., Backx, P.H., Yagil, Y., Penninger, J.M., 2002. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 417, 822–828.

Fujita, K., Matsumura, Y., Miyazaki, Y., Takaoka, M., Morimoto, S., 1995. Role of endothelin-1 in hypertension induced by long-term inhibition of nitric oxide synthase. Eur. J. Pharmacol. 280, 311–316.

Fujita, K., Matsumura, Y., Miyazaki, Y., Hashimoto, N., Takaoka, M., Morimoto, S., 1996. ETA receptor-mediated role of endothelin in the kidney of DOCA-salt hypertensive rats. Life Sci. 58, L1–L7.

Gallego, M.S., Ling, B.N., 1996. Regulation of amiloride-sensitive Na+ channels by endothelin-1 in distal nephron cells. Am. J. Physiol. 271, F451–F460.

Gariepy, C.E., Ohuchi, T., Williams, S.C., Richardson, J.A., Yanagisawa, M., 2000. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. J. Clin. Invest. 105, 925–933.

Giachelli, C.M., Pichler, R., Lombardi, D., Denhardt, D.T., Alpers, C.E., Schwartz, S.M., Johnson, R.J., 1994. Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. Kidney Int. 45, 515–524.

Gilmore, E.S., Stutts, M.J., Milgram, S.L., 2001. SRC family kinases mediate epithelial Na+ channel inhibition by endothelin. J. Biol. Chem. 276, 42610–42617.

Grunder, S., Zagato, L., Yagil, C., Yagil, Y., Sassard, J., Rossier, B.C., 1997. Polymorphisms in the carboxy-terminus of the epithelial sodium channel in rat models for hypertension. J. Hypertens. 15, 173–179.

Johnson, R.J., Gordon, K.L., Suga, S., Duijvestijn, A.M., Griffin, K., Bidani, A., 1999. Renal injury and salt-sensitive hypertension after exposure to catecholamines. Hypertension 34, 151–159.

Kassab, S., Miller, M.T., Novak, J., Reckelhoff, J., Clower, B., Granger, J.P., 1998. Endothelin-A receptor antagonism attenuates the hypertension and renal injury in Dahl salt-sensitive rats. Hypertension 31, 397–402.

Kreutz, R., Struk, B., Rubattu, S., Hubner, N., Szpirer, J., Szpirer, C., Ganten, D., Lindpaintner, K., 1997. Role of the α-, β-, and γ-subunits of epithelial sodium channel in a model of polygenic hypertension. Hypertension 29, 131–136.

Kreutz, R., Kovacevic, L., Schulz, A., Rothermund, L., Ketteler, M., Paul, M., 2000. Effect of high NaCl diet on spontaneous hypertension in a genetic rat model with reduced nephron number. J. Hypertens. 18, 777–782.

Matsumura, Y., Hashimoto, N., Taira, S., Kuro, T., Kitano, R., Ohkita, M., Opgenorth, T.J., Takaoka, M., 1999. Different contributions of endothelin-A and endothelin-B receptors in the pathogenesis of deoxycorticosterone acetate-salt-induced hypertension in rats. Hypertension 33, 759–765.

Matsumura, Y., Kuro, T., Konishi, F., Takaoka, M., Gariepy, C.E., Yanagisawa, M., 2000. Enhanced blood pressure sensitivity to DOCA-salt treatment in endothelin ET(B) receptor-deficient rats. Br. J. Pharmacol. 129, 1060–1062.

Nakov, R., Pfarr, E., Eberle, E., 2002. Darusentan an effective endothelin A receptor antagonist for the treatment of hypertension. Am. J. Hypertens. 15, 583–589.

Ohuchi, T., Yanagisawa, M., Gariepy, C.E., 2000. Renal tubular effects of endothelin-B receptor signaling. Its role in cardiovascular homeostasis and extracellular volume regulation. Curr. Opin. Nephrol. Hypertens. 9, 435–439.

Orth, S.R., Esslinger, J.P., Amann, K., Schwarz, U., Raschack, M., Ritz, E., 1998. Nephroprotection of an ET(A)-receptor blocker (LU 135252) in salt-loaded uninephrectomized stroke-prone spontaneously hypertensive rats. Hypertension 31, 995–1001.

Pollock, D.M., 2000. Renal endothelin in hypertension. Curr. Opin. Nephrol. Hypertens. 9, 157–164.

Pollock, D.M., Derebail, V.K., Yamamoto, T., Pollock, J.S., 2000. Combined effects of AT(1) and ET(A) receptor antagonists, candesartan, and A-127722 in DOCA-salt hypertensive rats. Gen. Pharmacol. 34, 337–342.

Rothermund, L., Luckert, S., Kossmehl, P., Paul, M., Kreutz, R., 2001. Renal endothelin ET(A)/ET(B) receptor imbalance differentiates salt-sensitive from salt-resistant spontaneous hypertension. Hypertension 37, 275–280.

Rothermund, L., Vetter, R., Dieterich, M., Kossmehl, P., Gögebakan, Ö., Yagil, C., Yagil, Y., Kreutz, R., 2002. Endothelin-A receptor blockade prevents left ventricular hypertrophy and dysfunction in salt-sensitive experimental hypertension. Circulation 106, 2305–2308.

Schiffrin, E.L., 1999. Role of endothelin-1 in hypertension. Hypertension 34, 876–881.

Suga, S.I., Phillips, M.I., Ray, P.E., Raleigh, J.A., Vio, C.P., Kim, Y.G., Mazzali, M., Gordon, K.L., Hughes, J., Johnson, R.J., 2001. Hypokalemia induces renal injury and alterations in vasoactive mediators that favor salt sensitivity. Am. J. Physiol. Renal Physiol. 281, F620–F629.

Verhagen, A.M., Rabelink, T.J., Braam, B., Opgenorth, T.J., Grone, H.J., Koomans, H.A., Joles, J.A., 1998. Endothelin A receptor blockade alleviates hypertension and renal lesions associated with chronic nitric oxide synthase inhibition. J. Am. Soc. Nephrol. 9, 755–762.

Weinstock, M., Schorer-Apelbaum, D., Ben-Ishay, D., 1984. Baroreflex sensitivity and susceptibility to systolic hypertension induced by DOCA-salt in the Sabra rat. Am. J. Physiol. 246, H448–H452.

Xie, Y., Sakatsume, M., Nishi, S., Marita, I., Arakawa, M., Gejyo, F., 2001. Expression, roles, receptors, and regulation of osteopontin in the kidney. Kidney Int. 60, 1645–1657.

Yagil, C., Katni, G., Rubattu, S., Stolpe, C., Kreutz, R., Lindpaintner, K., Ben-Ishay, D., Yagil, Y., 1996. Development, genotype and phenotype

of the newly inbred sabra hypertension prone (SBH/y) and resistant (SBN/y) rat model of salt sensitivity and resistance. J. Hypertens. 14, 1175–1182.

Yagil, C., Sapojnikov, M., Kreutz, R., Katni, G., Lindpaintner, K., Ganten, D., Yagil, Y., 1998. Salt susceptibility maps to chromosomes 1 and 17 with sex specificity in the sabra experimental model of hypertension. Hypertension 31, 119–124.

Yagil, C., Sapojnikov, M., Kreutz, R., Zürcher, H., Ganten, D., Yagil, Y., 1999. Role of chromosome X in the Sabra rat model of salt-sensitive hypertension. Hypertension 33, 261–265.