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Abstract: This study investigated whether intrarenal endothelin-1(ET-1) contributes to sodium excretion in aged rats. Metabolic function studies were performed in male Wistar rats (3 and 24 months) treated with placebo or the orally active ET(A) receptor antagonist darusentan (20 mg/kg/d) for 4 weeks. Mean arterial pressure was measured using an intra-arterial catheter. Electrolytes, aldosterone levels, renin activity, and angiotensin converting enzyme activity were determined in plasma, and mRNA expression of epithelial sodium channel (ENaC) and Na(+), K(+)-ATPase subunits was measured in the renal cortex and medulla. Aging was associated with a marked decrease in urinary excretion of sodium, chloride, and potassium (all $P < 0.001$) as well as renin activity ($P < 0.05$), but had no significant effect on gene expression of ENaC or Na(+), K(+)-ATPase subunits. In aged rats, darusentan treatment increased ion excretion ($P < 0.05$), reduced cortical gene expression of alphaENaC and alpha(1)-Na(+), K(+)-ATPase (both $P < 0.05$), and increased plasma aldosterone levels ($P < 0.01$). These data demonstrate a decrease of sodium and potassium excretion in aged rats, changes that are partly sensitive to ETA receptor blockade. Treatment with darusentan also reduced cortical expression of alphaENaC and alpha(1)-Na(+), K(+)-ATPase and increased plasma aldosterone levels independently of blood pressure, electrolytes, renin activity, or angiotensin converting enzyme activity. These findings may provide new pathogenetic links between aging and sodium sensitivity.

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Endothelin ET_A Receptor Blockade With Darusentan Increases Sodium and Potassium Excretion in Aging Rats

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This study investigated whether intrarenal endothelin-1 (ET-1) contributes to sodium excretion in aged rats. Metabolic function studies were performed in male Wistar rats (3 and 24 months) treated with placebo or the orally active ET_A receptor antagonist darusentan (20 mg/kg/d) for 4 weeks. Mean arterial pressure was measured using an intra-arterial catheter. Electrolytes, aldosterone levels, renin activity, and angiotensin converting enzyme activity were determined in plasma, and mRNA expression of epithelial sodium channel (ENaC) and Na^+, K^+-ATPase subunits was measured in the renal cortex and medulla. Aging was associated with a marked decrease in urinary excretion of sodium, chloride, and potassium (all $P < 0.001$) as well as renin activity ($P < 0.05$), but had no significant effect on gene expression of ENaC or Na^+, K^+-ATPase subunits. In aged rats, darusentan treatment increased ion excretion ($P < 0.05$), reduced cortical gene expression of αENaC and α_1-Na^+, K^+-ATPase (both $P < 0.05$), and increased plasma aldosterone levels ($P < 0.01$). These data demonstrate a decrease of sodium and potassium excretion in aged rats, changes that are partly sensitive to ET_A receptor blockade. Treatment with darusentan also reduced cortical expression of αENaC and α_1-Na^+, K^+-ATPase and increased plasma aldosterone levels independently of blood pressure, electrolytes, renin activity, or angiotensin converting enzyme activity. These findings may provide new pathogenetic links between aging and sodium sensitivity.

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Endothelin-1[1–21] (ET-1) is one of the most potent vasoconstrictor peptides known[1,2] and the predominant isoform of the ET peptide family. It has been implicated in the pathogenesis of cardiovascular and renal disease (reviewed in Refs. 3, 4), including salt-sensitive hypertension.[5] Two human receptors for ET-1 have been identified and cloned: the ET_A receptor which is located on vascular smooth muscle cells mediating vasoconstriction, and the ET_B receptor which is predominantly located on endothelial cells mediating vasodilation (reviewed in Ref. 3). A number of orally active antagonists for these receptors have been developed and experimental studies demonstrated their beneficial effect in different models of cardiovascular and renal disease, partly or completely independent of blood pressure.[6–11]

Aging is an important and independent risk factor for renal and cardiovascular disease.[12] In the aging kidney, changes occur with regard to glomerular structure and function, blood flow, and excretion of ions and drugs (reviewed in Ref. 13). We[14,15] and others[16] have previously shown that renal ET-1 expression increases in the aging kidney and that it contributes to glomerulosclerosis.[11] Although endothelins are known to regulate several functions in renal physiology, including vascular tone, cell proliferation, and diuresis (reviewed in Ref. 4), its role for ion excretion with aging is still unclear. Impairment in sodium excretion (or increased sodium reabsorption, respectively) may result in elevated blood pressure frequently defined as "salt-sensitive" hypertension; however, the underlying mechanisms of salt sensitivity are not fully understood.[17] The incidence of sodium sensitivity increases with age[18] and is associated with a high risk for end-organ damage and cardiovascular events.[19] Moreover, there is evidence for a relationship between sodium sensitivity and cardiovascular mortality in normotensive patients even before the onset of hypertension.[20]

The objective of the present study was therefore to test the hypothesis whether intrarenal ET-1 contributes to the regulation of ion excretion in aged rats by using the

orally active ET_A receptor antagonist darusentan. As sodium reabsorption in the distal nephron primarily occurs via the apical epithelial sodium channel (ENaC) maintained by the basolateral Na^+, K^+-ATPase, the effects of aging and/or ET_A receptor blockade on the expression of these genes in the renal cortex and medulla were also investigated.

METHODS

Animal Studies

Male Wistar rats (IFFA Credo/Charles River, L-Arbesle, France) were subjected to 24-hour metabolic studies at the age of 2 and 23 months as described.[11] Briefly, animals were placed in individual metabolic cages and urine was collected after a 24-hour acclimatization period. Urine was centrifuged, stored at $-80°C$, and determination of urinary parameters was performed on an autoanalyzer (model 917, Roche/Hitachi, Basel, Switzerland). Animals were randomized to treatment for 4 weeks with or without the orally active ET_A receptor antagonist darusentan (formerly LU135252, 182-fold selectivity for ET_A over ET_B; 20 mg/kg/d, Knoll AG, Ludwigshafen, Germany; $n = 8$ to 9 per group) in drinking water as described.[11] Food intake (standard rodent chow) and water intake was monitored throughout the study. At the end of treatment, metabolic cage studies were repeated. Animals were anesthetized (thiopental, 50 mg/kg body weight, IP), venous blood samples were obtained (centrifuged at 4°C and 5000 rpm for 15 min and plasma stored at $-80°C$), and animals were killed by exsanguination. Kidneys were removed, decapsulated, immediately snap-frozen in liquid nitrogen, and kept at $-80°C$. Mean arterial blood pressure measurements were performed using an intra-arterial catheter as described.[11] Experiments were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the NIH.

Measurement of Sodium, Chloride, and Potassium in Plasma

Electrolytes were measured using a Cobas Integra 800 (Roche Diagnostics, Rotkreuz, Switzerland) by standard procedures recommended by the International Federation of Clinical Chemistry.[21]

Measurement of Aldosterone Levels, Renin Activity, and Angiotensin Converting Enzyme Activity in Plasma

Plasma aldosterone levels (nmol/L) were quantified using a direct radioimmunoassay (RIA) with a specific antiserum and ^{3}H-labeled aldosterone as previously described.[22] Plasma renin activity (μg/L/h) was quantified by RIA (GammaCoat, DiaSorin Inc, MN), which involves an initial incubation of plasma to generate angiotensin I, followed by the quantification of angiotensin I by RIA. Angiotensin converting enzyme (ACE) activity (U/L) was quantified by a radio-enzymatic assay (Bühlmann Laboratories AG, Allschwil, Switzerland).

Renal Gene Expression Studies

Renal cortex and medulla were separated as described previously.[9] Total RNA was extracted from tissues using the silica-based RNeasy Minikit (Qiagen, Hilden, Germany). Purity of RNA was controlled by RT(−) reactions (PCR with nontranscribed RNA). RNA was reverse-transcribed with the Omniscript RT kit (Qiagen, Hilden, Germany). Real-time quantitative PCR was used as described[11] to quantify expression of genes encoding rat αENaC, βENaC, γENaC, α_1-Na^+, K^+-ATPase, and γ-Na^+, K^+-ATPase. For standardization, expression of the gene encoding for rat 18s RNA was used (house-keeping gene). The following primers were used: 5′-ACC GCT TCC ATT ACA TCA ACA TTC-3′ (forward), 5′-GTG GAA CTT GGA ATA ATT CGC CTG-3′ (reverse) for amplification of an αENaC specific cDNA fragment; 5-GCC ATG TGG TTC CTG CTC AC-3′ (forward), 5′-GCT CAG GTA GGT CTG GAT GAA GA-3′ (reverse) for amplification of a βENaC specific cDNA fragment; 5′-CAA AGC CAA GGA TTG TTG GGC-3′ (forward), 5′-AGG TCA TCG TCC GTA TCC AGA G-3′ (reverse) for amplification of a γENaC specific cDNA fragment; 5′-TGG GCA TTA TCT CAG AAG GTA ACG-3′ (forward), 5′-TCA CCT GGT TCA CTG GAA TGT TG-3′ (reverse) for amplification of an α_1-Na^+, K^+-ATPase specific cDNA fragment and 5′-AAA CCG TCC GCA AAG GAG GC-3′ (forward), 5′-CCG TCA CAG CTC ATC TTC ATT GAC-3′ (reverse) for amplification of a γ-Na^+, K^+-ATPase specific cDNA fragment, and rat 18s ribosomal RNA (primers 5′-ACA CGG ACA GGA TTG ACA GAT TG-3′ (forward), 5′-CAG ACA AAT CGC TCC ACC AAC T-3′ (reverse). Two-step PCR was performed with an iQ SYBR Supermix PCR kit (Bio-Rad, Switzerland) as follows: activation of the hot start *Taq* polymerase for 3 minutes (95°C), followed by 45 cycles of denaturation at 95°C for 15 seconds (step 1), and annealing and extension at 60°C for 1 minute (step 2). Fluorescence was detected at the end of each extension step. Identity and specificity of amplicons were confirmed by agarose gel electrophoresis, melting curve analysis, and sequencing (Microsynth, Balgach, Switzerland). Gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.[11]

Statistical Analysis

Data are given as mean ± SEM and n equals the number of animals. For multiple comparisons, results were analyzed using ANOVA, followed by Bonferroni's correction. For comparisons between 2 values, the unpaired or paired t test were used when appropriate. A P value < 0.05 was considered significant.

RESULTS

Physiologic Parameters

During the study period of 4 weeks, body weight increased in young but not in old rats ($P < 0.001$, Table 1). Aging was associated with a decrease in food intake (31 ± 5 vs. 54 ± 1 g/kg/d, $P < 0.001$ vs. young),

TABLE 1. Physiological Parameters

Age	Young		Old	
Treatment	Control	Darusentan	Control	Darusentan
Body weight, week 0 (g)	318 ± 3	322 ± 4	652 ± 24*	636 ± 18*
Body weight, week 4 (g)	436 ± 5†	452 ± 8†	631 ± 36*	643 ± 18*
Water intake (g/kg/d)	72 ± 4	71 ± 3	35 ± 5*	39 ± 5*
Urine excretion (g/kg/d)	36 ± 3	30 ± 3	18 ± 2*	23 ± 2
Mean arterial pressure (mm Hg)	102 ± 3	101 ± 2	101 ± 1	103 ± 3

Effects of aging and darusentan treatment on body weight, water intake, urine excretion, and mean arterial pressure. Data are means ± SEM, n = 8 to 9 animals.
*$P < 0.001$ versus young.
†$P < 0.001$ versus week 0.

water intake, and urine excretion (both $P < 0.001$ vs. young, Table 1) whereas mean arterial pressure was not affected by aging (ns, Table 1). Darusentan treatment had no effect on food intake (56 ± 2 vs. 54 ± 1 and 38 ± 4 vs. 31 ± 5 g/kg/d, ns), body weight, water intake, urine excretion, and mean arterial pressure in young or aged animals (all ns, Table 1).

Renal Function Studies

In aged animals, a decrease in the absolute excretion of sodium ions (2.6 ± 0.2 vs. 5.8 ± 0.3 mmol/kg/24 h), chloride ions (2.2 ± 0.2 vs. 6.0 ± 0.2 mmol/kg/24 h), and potassium ions was observed (5.5 ± 0.4 vs. 12.5 ± 0.3 mmol/kg/24 h, all $P < 0.001$ vs. young, Figs. 1A–C, left panels). In aged but not in young rats, darusentan treatment increased the excretion of sodium (+35%), chloride (+46%), and potassium (+27%, all $P < 0.05$ vs. untreated, Figs. 1A–C, left panels). Changes in ion excretion before and after darusentan treatment were also observed intraindividually in aged rats: absolute excretion of sodium increased from 3.0 ± 0.3 to 3.5 ± 0.2 mmol/kg/24 h, chloride from 2.6 ± 0.3 to 3.3 ± 0.3 mmol/kg/24 h, and potassium from 6.1 ± 0.4 to 7.0 ± 0.4 mmol/kg/24 h (all $P < 0.01$, paired t test, Figs. 1A–C, right panels).

Plasma Levels of Sodium, Chloride, and Potassium

Aging or treatment with darusentan had no effect on plasma electrolytes (all ns, Table 2).

Plasma Aldosterone Levels, Renin Activity, and ACE Activity

Aldosterone plasma levels were similar in untreated animals. However, darusentan treatment increased plasma aldosterone levels by 43% in aged but not in young rats (from 2.3 ± 0.1 to 3.3 ± 0.3 nmol/L, $P < 0.01$ vs. untreated, Fig. 2A). Plasma renin activity was reduced by more than 75% with aging (5.3 ± 1.3 vs. 22.6 ± 3.7 μg/L/h, $P < 0.05$, Fig. 2B) and unaffected by treatment. ACE activity was unaffected by aging or darusentan treatment (Fig. 2C).

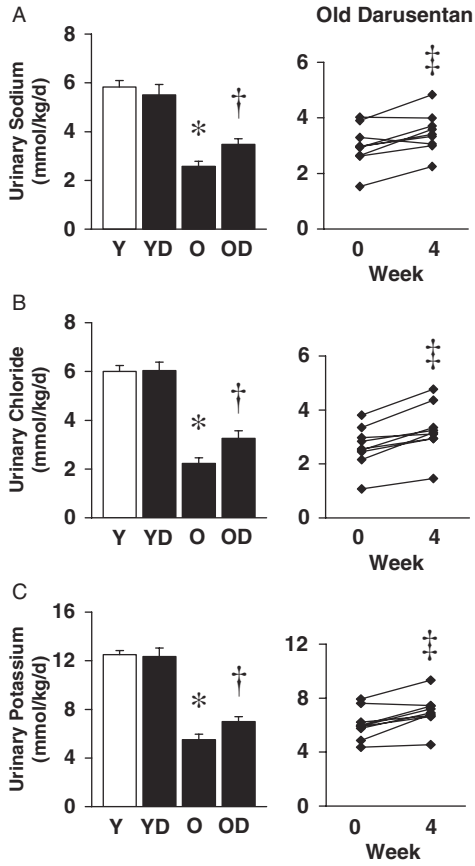

FIGURE 1. Effects of aging and darusentan treatment on absolute urinary excretion of sodium (A), chloride (B), and potassium (C). Right panels: paired data of individual animals. Effects of darusentan treatment on absolute urinary excretion of sodium (A), chloride (B), and potassium (C) in aged rats after 4 weeks. Y indicates young control; YD, young darusentan; O, old control; OD, old darusentan. Every column represents n = 8 to 9 animals. Data are means ± SEM. *$P < 0.001$ versus young, †$P < 0.05$ versus old control, ‡$P < 0.01$ versus week 0.

Renal Gene Expression of ENaC- and Na^+, K^+-ATPase Subunits

Marked differences with regard to absolute expression levels between subunits of Na^+, K^+-ATPase and ENaC were noted, with expression being highest for α_1-Na^+, K^+-ATPase and αENaC (data not shown). Expression of γ-Na^+, K^+-ATPase was higher in the

TABLE 2. Plasma Electrolytes

Age	Young		Old	
Treatment	Control	Darusentan	Control	Darusentan
Sodium (mmol/L)	146 ± 0	147 ± 1	147 ± 1	148 ± 1
Chloride (mmol/L)	106 ± 1	106 ± 1	107 ± 1	109 ± 1
Potassium (mmol/L)	4.6 ± 0.3	4.9 ± 0.2	4.1 ± 0.3	4.6 ± 0.2

Effects of aging and darusentan treatment on plasma values of sodium, chloride, and potassium. Data are means ± SEM, n = 4 to 7 animals.

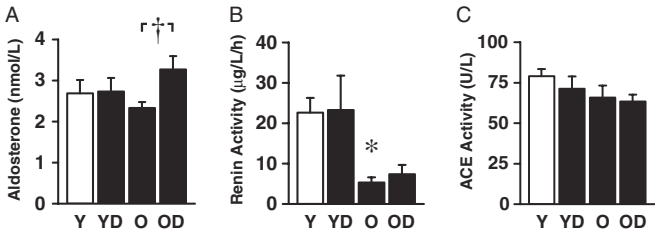

FIGURE 2. Effects of aging and darusentan treatment on plasma aldosterone levels (A), renin activity (PRA, B), and ACE activity (C). Y indicates young control; YD, young darusentan; O, old control; OD, old darusentan. Columns represent n = 7 animals (A, B) and n = 4 to 7 (C), respectively. Data are means ± SEM. *$P < 0.05$ versus young, †$P < 0.01$ versus old control.

medulla than in the cortex of young animals ($P < 0.05$, data not shown), whereas the other subunits showed comparable expression levels between the medulla and cortex. Surprisingly, aging per se had no significant effect on gene expression; however, there was a trend toward higher expression of αENaC in the medulla of aged animals ($P = 0.15$ vs. young, Fig. 3A, right panel). Darusentan treatment reduced gene expression of αENaC (−72%, $P < 0.05$, Fig. 3A, left panel) and α_1-Na^+, K^+-ATPase in the cortex of old rats (−50%, $P < 0.05$, Fig. 3B, left panel).

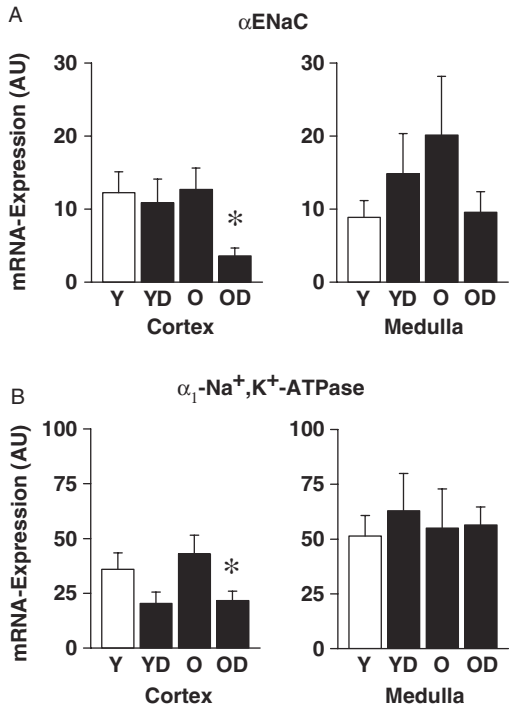

FIGURE 3. Effects of aging and darusentan treatment on renal mRNA expression of αENaC (A) and α_1-Na^+, K^+-ATPase (B) in the cortex (left panels) and medulla (right panels). Y indicates young control; YD, young darusentan; O, old control; OD, old darusentan. Every column represents n = 6 to 9 animals. Data are means ± SEM. *$P < 0.05$ versus old control.

DISCUSSION

This study demonstrates that aging leads to a marked reduction of sodium, potassium, and chloride excretion in otherwise healthy and normotensive rats, which was reversed in part after treatment with an orally active endothelin receptor antagonist. As no effects were observed in young animals, these data suggest a role for the ET_A receptor contributing to renal excretory function in the aged kidney. The results further show that ET_A receptor blockade reduces cortical αENaC as well as α_1-Na^+, K^+-ATPase expression and increases plasma aldosterone levels in aged rats only. These effects occured independently of mean arterial pressure, plasma electrolytes, or activity of circulating renin and ACE.

In previous studies, we have demonstrated that renal ET-1 expression increases in aged rodents[14,15] and that ET-1 contributes to age-dependent glomerulosclerosis.[11] Studies in ET-transgenic mice showed age-dependent glomerulosclerosis, interstitial fibrosis, and decreased creatinine clearance and/or glomerular filtration rate, respectively.[23,24] Interestingly, ET-transgenic animals do not spontaneously develop hypertension; however, one of the transgenic lines described by Hocher et al[23] was characterized by high intrarenal ET-1 expression and also showed a marked decrease in sodium excretion with aging. The finding that sodium excretion decreases with aging might have been partly influenced by lower food intake in aged rats. However, and in line with the findings of the present study, a marked decrease in sodium excretion was observed in aged females of the same strain.[25,26] As one of these studies was performed after an overnight fasting period,[26] and changes in sodium homeostasis in the rat require only hours,[27] a decreased ion excretion in aging rats is unlikely to be determined by food intake alone. Renal aging has been associated with a failure to conserve sodium and therefore potential "salt-wasting;"[13] interestingly, salt-sensitive hypertension in humans is also associated with impaired sodium excretion and suppressed renin levels.[17] In this context it is interesting to note that decreases in urinary potassium excretion, as seen in our study, have been most recently suggested to play a role in sodium sensitivity in blacks.[28]

Compatible with our findings that show improved sodium excretion after ET_A receptor blockade, ET-1 infusion decreases sodium excretion in healthy volunteers via activation of ET_A receptors.[29,30] Two previous animal studies have used the nonselective ET receptor antagonist bosentan acutely to determine kidney function. Consistent with our observations, Greenfeld et al[31] reported an increased excretion of sodium in Sprague-Dawley rats of different age groups after blockade of endogenous ET-1, whereas in a study performed in Wistar rats by Hocher et al[32] bosentan decreased sodium excretion on top of coinfusion of ET-1. In these studies[31,32] and in our previous study,[11] ET receptor blockade had no effect on blood pressure, glomerular filtration rate, renal blood flow, or urine flow. Indeed, the data of the present study indicate that blocking endogen-

ous ET_A receptor activation was unrelated to urine excretion; additionally, treatment had no effect on body weight or food and water intake. This is in line with 2 studies also reporting no effect of ET receptor blockade on food and water intake or urine volume in adult Sprague-Dawley rats.[33,34] However, we cannot exclude the possibility that additional, nonrenal mechanisms such as effects of ET on the central nervous system were also involved in the natriuretic effects of darusentan.[35]

With regard to the role of ET-1 for sodium homeostasis, the data available up to now have been conflicting. In vivo studies using systemic infusion suggest that ET-1 may decrease or increase sodium excretion through ENaC activation depending on the dose of ET-1 (reviewed in Ref. 4). A bimodal function of ET-1 for ENaC regulation in distal nephron cells in vitro was also demonstrated by Gallego and Ling.[36] These investigators found that nanomolar concentrations of ET-1 activate ENaC via the ET_A receptor, resulting in sodium reabsorption in the collecting duct, whereas lower concentrations inhibit ENaC by ET_B receptor activation. Such a mechanism could provide a possible explanation for our in vivo findings showing that impaired excretion of sodium chloride was reversed in part after selective ET_A blockade. The data of the present study further suggest a functional role for endogenous ET-1 in the aged kidney that may depend on local concentrations of ET-1 peptide. Thus, in the aged kidney increased local levels of endogenous ET[14,37] may promote sodium reabsorption, whereas blockade of the ET_A receptor interferes with ENaC activation. This notion would also be supported by the observation that darusentan had no effect in young rats on any of the parameters investigated. Moreover, we propose that, during darusentan treatment of aged rats, an ET_B-mediated inhibitory effect on ENaC and sodium reabsorption becomes activated, as increased intrarenal levels of the ET_B agonists ET-1 and ET-3[14,37] may still activate the ET_B receptor in the presence of selective ET_A receptor blockade. Indeed, studies in ET_B receptor-deficient animals as well as functional studies have provided substantial evidence supporting a role of this receptor in salt-sensitivity by regulating sodium excretion via ENaC.[32,38–41] In addition to effects on ENaC, ET-1 also inhibits Na^+, K^+-ATPase activity in the collecting duct[42] and thus affects transepithelial sodium transport.[43] Na^+, K^+-ATPase importantly controls transepithelial sodium transport and has been localized in most nephron cells. In the rat kidney, α_1-Na^+, K^+-ATPase is the predominant catalytic and transporting unit whereas β and γ-subunits seem to have a modulatory role.[44] A study investigating Na^+, K^+-ATPase activity in the proximal convoluted tubule found a decrease of Na^+, K^+-ATPase activity with aging,[45] whereas another study reported no such effects.[46]

To determine whether the effects of aging and/or ET receptor blockade affect intrarenal gene expression of sodium-regulating proteins, molecular analyses were performed in renal cortex and medulla. Surprisingly, and despite the changes in ion excretion, we found that in the renal cortex and medulla aging per se had no effect on expression levels of the genes investigated. However, a down-regulation of αENaC and α_1-Na^+, K^+-ATPase gene expression was observed after darusentan treatment only in the cortex of aged rats; interestingly, this effect was unrelated to mean arterial pressure, circulating renin, or ACE activity. The underlying mechanisms for this regulation are currently unknown but may include interactions between proteinuria and natriuresis,[47] as we have previously shown that ET_A receptor blockade improves age-dependent proteinuria by reversing podocyte injury.[11] The benefit of ENaC-inhibition in blacks with hypertension, who are often resistant to standard antihypertensive therapy,[48] has been most recently demonstrated.[49] Alternatively, mechanisms by which darusentan affects sodium excretion may include effects of serum-regulated and glucocorticoid-regulated kinase-1, which mediates ENaC trafficking and is stimulated by ET-1. This effect of glucocorticoid-regulated kinase-1 is comparable to that of aldosterone and sensitive to ET_A receptor blockade.[50,51] As we detected a down-regulation of αENaC gene expression after darusentan treatment only in the cortex and as ENaC subunits are known to be localized among others in the cortical collecting duct,[52] the effects of darusentan might also involve suppression of ENaC mRNA in the cortical collecting duct. Furthermore, the observed down-regulation of the αENaC gene is likely to contribute to sodium excretion as the gene product is rate limiting for the assembly of the mature ENaC complex.[53] Although the data of the present study using whole kidney tissue of cortex and medulla suggest the possibility that cortical ENaC and Na^+, K^+-ATPase expression may contribute to renal sodium handling in the aging kidney, the results of the present study are limited by the fact that only mRNA expression was investigated and that protein abundance or enzyme activity might not have been equally affected by darusentan treatment.

Sodium excretion in the collecting duct via ENaC in addition to ET-1 is regulated by aldosterone[54]; however, other humoral factors such as renin, vasopressin,[54] and nitric oxide (NO)[55] also play a role. Regarding NO, we have previously shown that in the presence of increasing ET-1 expression in the aging kidney, renal tissue levels of stable metabolites of NO decrease[14] as does basal and stimulated endothelial NO bioactivity.[56] Given that NO is a potent inhibitor of both ET-1[57] and also of sodium reabsorption,[55] age-dependent decreases in NO bioactivity may further reduce sodium excretion, and thereby aggravate the effect of ET-1 in aged rats. Interestingly, and in line with what has been shown in humans,[58] aging in the present study was associated with a more than 75% decrease in renin activity, but not aldosterone plasma levels. An unexpected result of the present study was the finding that ET_A receptor blockade increased aldosterone plasma levels in aged but not in young rats. This effect of darusentan could be related to selective ET_B receptor stimulation in the rat[59,60] and, possibly, the effects of the drug on sodium excretion might have been even more

pronounced if aldosterone levels had been unaffected. An expected rise in blood pressure with increased aldosterone levels may, however, be prevented by ET_A receptor blockade.[61] Additionally, ET-mediated increases in aldosterone secretion could be responsible for the increased potassium excretion observed in darusentan-treated aged animals.

CONCLUSIONS

The findings of the present study indicate that aged in otherwise healthy and normotensive rats is associated with changes in ion secretion and plasma renin activity. In aged but not in young rats, ion excretion and aldosterone plasma levels increased after 4 weeks of ET_A receptor blockade. These changes were associated with the inhibition of gene expression of 2 important regulators of sodium homeostasis in the renal cortex, αENaC and α_1-Na^+, K^+-ATPase. These findings may be of possible relevance for the pathogenesis of sodium sensitivity, which increases with aging and is associated with an activation of the ET system in animals and humans.

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