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Low Sodium Modifies the Vascular Effects of ACE Inhibitor Therapy in Healthy Rats

Menno JA Kocks[1,2], Simone Gschwend[1], Dick de Zeeuw[1,2], Gerjan Navis[1,2], Hendrik Buikema[1]

Department of Clinical Pharmacology, University of Groningen[1], and the department of Internal Medicine (section Nephrology), University Hospital Groningen[2], Groningen, The Netherlands

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Running title

Effect of dietary sodium on vascular effects of ACEi

Address for correspondence: H. Buikema, Ph.D.

Department of Clinical Pharmacology, University of Groningen

Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Phone: +31 50 3632810 Fax: +31 50 3632812

Email: h.buikema@med.rug.nl

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List of Abbreviations: RAS: Renin-Angiotensin System

ACE: Angiotensin-Converting Enzyme

CON: Control group

CON-LIS: Lisinopril treated group

LS: Vehicle treated group during low dietary sodium

LS-LIS: Lisinopril treated group during low dietary sodium

PE: Phenylephrine

ACh: Acetylcholine

SNP: Sodium Nitroprusside

COX: Cyclooxygenase

Subject: Cardiovascular

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Abstract

Low dietary sodium (LS) increases the effect of ACE inhibitor therapy in patients and experimental models, but mechanisms underlying this enhanced efficacy are largely unknown. Because the benefits of ACE inhibition are mediated to a considerable extent by their effect on the vasculature, we studied whether low sodium alters the vascular effects of ACE inhibition. Baseline functional and morphological characteristics, and endothelium-dependent and -independent dilatory responses were studied in isolated perfused small intra-renal and mesenteric arteries obtained from control rats (CON), rats on LS, lisinopril treated rats (CON-LIS) or rats treated with lisinopril during LS (LS-LIS). We found, first, that LS-LIS as compared to CON-LIS enhances blood pressure reduction. Second, interlobar renal arteries had increased lumen diameter and reduced adrenergic contractility in CON-LIS compared to CON, without additional effects of LS. In contrast, mesenteric arteries were not altered in CON-LIS compared to CON, but became triggered for increased myogenic and adrenergic constriction in LS-LIS. Third, LS-LIS decreased ACh-induced vasodilation in both mesenteric and renal arteries compared to CON-LIS. During the latter condition opposite prostaglandins are involved in the endothelial function of the two different vascular beds; i.e. increased involvement of contractile prostaglandins in ACh-induced vasodilatation in renal arteries, versus dilatory prostaglandins in mesenteric arteries. Whether cause or consequence of the enhanced blood pressure response, our data demonstrate a modifying effect of dietary sodium on vascular effects of ACE inhibition. These findings provide a rationale for further studies addressing the mechanism-of-actions of our therapies to find additional strategies to improve therapy response.

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Introduction

Intervention in the renin-angiotensin system (RAS) by angiotensin converting enzyme (ACE) inhibitors has proven to be an effective strategy to improve renal and cardiovascular prognosis in different patient populations. The response to ACE inhibition is modified by sodium intake, with a blunted response during high sodium intake, and an enhanced response during dietary sodium restriction. This occurs irrespective of the underlying disorder and applies to the effect on blood pressure, renal hemodynamic response, and proteinuria (Heeg et al., 1989; Buter et al., 1998; Teravainen et al., 1997), in experimental conditions and as well as in man (Wapstra et al., 1996; Wing et al., 1998; Navis et al., 1987).

Although ACE inhibitors have been studied extensively, the mechanism of the modifying effect of sodium intake on their efficacy is not well understood. The effects of ACE inhibition are believed to result from their hemodynamic actions, as well as from pressure-dependent and -independent effect on the vessel wall. In this respect, many studies showed improved vessel wall structure and dimension, and improved endothelial function in cardiovascular disease after chronic ACE inhibitor therapy (Mancini, 2000). We hypothesized that dietary sodium intake modifies the vascular effects of maintenance treatment with ACE inhibitors.

To address this hypothesis, isolated perfused preparations of small intra-renal and mesenteric rat artery were studied for baseline functional and morphological vessel characteristics, and endothelium-dependent and -independent dilatory responses after maintenance treatment with lisinopril with or without low dietary sodium. Because the heterogeneity of the vascular bed is well established, we studied two different vascular beds. Small renal interlobar arteries were studied because of the importance of the kidney as a target organ for ACE-inhibition (Wing, et al., 1998). In addition, small mesenteric arteries were studied because of the importance of this artery type in the regulation of total peripheral vascular resistance.

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Methods

Rat Studies

Male Wistar rats (250-300 g, Harlan, Zeist, The Netherlands) were housed under standard conditions at the animal facility of the University of Groningen and studied in accord with institutional and legislator regulations. After an adaptation period of one week, rats were allocated to one of four experimental groups (n=8-10 per group) receiving different treatments. The ACE inhibitor lisinopril (LIS, 75 mg/l) was given for a period of three weeks via tap water to rats either fed a control diet (Hope Farms, Woerden, The Netherlands) with modestly elevated sodium (CON-LIS, 2.0% NaCl) or a low sodium diet (LS-LIS, 0.05% NaCl), and comparisons were made to rats treated with vehicle (CON (2.0% NaCl) and LS (0.05% NaCl), respectively). Once per week rats were put in metabolic cages for collection of 24h urine samples and routine analyses of urinary sodium content. After prior training sessions to get accustomed with the experimental set-up, systolic blood pressure was determined in conscious animals at the end of the treatment period by means of the tail-cuff method using an automated multi-channel system (Life Science, Woodland Hills, California); a mean of three subsequent recordings was taken as the final value.

At sacrifice after three weeks of treatment, rats were anesthetized with 1.5% isofluran in N_2O/O_2 and blood samples were taken for determination of plasma ACE activity; i.e. hippuryl-his-leu cleavage method as previously described by (Hirsch et al., 1991). Intestines and kidneys were removed and put into cold Krebs solution. Third-order branches of the superior mesenteric artery and renal interlobar arteries of the right kidney were isolated from surrounding perivascular tissue in cold Krebs buffer solution.

Vascular Studies

Small renal interlobar arteries and small mesenteric arteries were transferred to an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT, USA) (Halpern et al., 1984). Artery segments were cannulated at both ends on glass micropipettes, secured, and the lumen of the vessel was filled with Krebs solution through the micropipettes as described earlier (Gschwend et al., 2002). Intraluminal pressure

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was set to 70 mmHg and held constant (blind sac) by a pressure servo system (Living System Instrumentation, Burlington, VT, USA). The vessel chamber was continuously re-circulated with warmed (37°C) and oxygenated (5% CO_2 in O_2) Krebs solution with a pH of 7.4. The vessel chamber was transferred to the stage of an inverted light microscope with a video camera attached to a viewing tube. The video dimension analyzer (Living System Instrumentation, Burlington, VT, USA) was used to analyze the signal obtained from the video image and to continuously register lumen diameter and wall thickness. Arteries were followed for development of myogenic tone at 70 mmHg and allowed to equilibrate for one hour in regular Krebs solution before being pre-constricted with phenylephrine (PE) (Gschwend et al., 2003).

Initially vessels were all stimulated with a fixed dose of PE ($3x10^{-7}$ mol/L) and the level of contraction was assessed. Thereafter - because this resulted in different contraction levels – the concentration of PE was slowly increased (varying from $3x10^{-7}$ to $3x10^{-6}$ mol/L) to finally obtain similar levels of pre-constriction (diameter reduction by 40±2% in mesenteric arteries, and by 37±1% in renal arteries). Pre-constricted vessels were then studied for endothelium-dependent relaxation by giving cumulative doses of acetylcholine (ACh; 10^{-8} mol/L - 10^{-4} mol/L) to the re-circulating bath.

To determine the contribution of vasoactive prostaglandins (PGs) the response to ACh was additionally studied as in the above but now in presence of the cyclooxygenase (COX) inhibitor indomethacin (10^{-5} mol/L) added to the organ bath 20 minutes prior to addition of ACh.

In a limited number of the arteries (n=4 for each group) - after endothelial function measurements - additional concentration-response curves to sodium nitroprusside (SNP, 10^{-9} – $3x10^{-4}$ mol/L) were obtained in pre-constricted arteries to account for dilative ability of arterial smooth muscle to NO.

Solutions and Drugs

Rats were treated with lisinopril supplied by Merck, Sharp & Dohme research Laboratories (Rahway, NJ USA).Vessel segments were superfused with Krebs solution containing (in mmol/L): 120.4 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.2 NaH_2PO_4, 11.5 glucose (Merck, Darmstadt, Germany). Acetylcholine chloride, L-Phenylephrine hydrochloride, Sodium nitroprusside dihydrate, and Indomethacin were obtained from

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Sigma-Aldrich Chemie B.V., The Netherlands. They were dissolved in de-ionized water and diluted with Krebs solution. Stock solution (10^{-2} mol/L) for indomethacin was prepared in 96% ethanol (<0.1% final organ bath concentration).

Data Analysis

Myogenic constriction was expressed as a percent constriction = 100 x [(Dbase – Dmyo)/Dbase], where D is the diameter before the development of myogenic tone (Dbase) or the diameter after the development of myogenic tone (Dmyo). Concentration-response curves to ACh and maximal relaxation (Emax) were expressed in percentage of preconstriction to PE. The Area Under each individual Curve (AUC) was determined (Sigma Plot, Jandell Scientific) and expressed in arbitrary units. The AUC was used to present total (individual) ACh dilation, and for subsequent analysis of differences in ACh dilation with and without indomethacin (Buikema et al., 2000). Data are expressed as mean ± standard error of the mean (SEM). Group-comparison was performed using one-way ANOVA, or repeated measures ANOVA in case of full concentration-response curves to ACh and SNP, and when appropriate corrected for multiple comparison by Duncan's multiple range test. Statistical differences were determined using student's paired or unpaired t-test, where appropriate. Significance was accepted at $P<0.05$.

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Results

Rat Characteristics

In conjunction with their diets, urinary sodium excretion was significantly higher in control rats (CON) than in low sodium fed rats (LS), and this was most pronounced in control rats treated with lisinopril (CON-LIS) (Table 1). Dietary sodium restriction per se did not affect rat body weight and systolic blood pressure in these healthy animals, which is in accord with the normal functioning of regulatory mechanisms of the renal-body fluid system for arterial pressure control. The effect of lisinopril on plasma ACE activity was comparable in both sodium groups. A significant reduction in body weight was observed only after treatment with lisinopril during low sodium intake. As anticipated, the reduction in systolic blood pressure after treatment with lisinopril was significantly more pronounced during low sodium intake as compared to control demonstrating the enhanced therapeutic efficacy of ACE inhibition during dietary sodium restriction.

Apart from urinary sodium excretion, the low dietary sodium per se as compared to the control diet had no significant effect on the parameters in Table 1, or on those investigated in the following sections. Therefore, for reasons of conciseness, the data from the low dietary sodium group were not presented hereafter.

Baseline Vessel Characteristics

Dietary sodium restriction per se had no significant effects on baseline morphologic and functional vascular properties in mesenteric resistance arteries and renal interlobar arteries (data not shown).

After treatment with lisinopril, renal arteries showed significantly increased lumen diameter at baseline and decreased PE-induced constriction. The effects were similar during both sodium regimens – i.e., the ACE inhibitor effect was not modified by dietary sodium restriction (Table 2).

In contrast to the renal arteries, baseline characteristics of mesenteric arteries were not affected by treatment with lisinopril. In combination with dietary sodium restriction, however, mesenteric arteries showed significantly increased myogenic tone development and increased PE-induced constriction (Table 2).

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The contribution of prostaglandins in the contractile response to PE in the two vascular beds is shown in figure 1. In renal arteries, incubation with indomethacin similarly reduced PE-induced constriction in all groups in such a way that lisinopril-induced group differences persisted; hence lisinopril-induced effects on PE-induced constriction persisted in presence of prostaglandin-inhibition (Figure 1A). In mesenteric arteries, presence of indomethacin reduced PE-induced constriction in all groups. This effect was most pronounced in LS-LIS, in which PE-induced constriction was profoundly increased as compared to the other groups (Figure 1B).

ACh induced dilation and the contribution of prostaglandins

Full concentration-response (CR-) curves to ACh and SNP in absence of indomethacin are given for individual groups in Figure 2. Endothelium-independent dilation to SNP did not differ between the groups - neither for mesenteric nor for renal arteries – implying that potential alterations at the level of vascular smooth muscle cell reactivity do not account for possible group-differences in ACh induced dilation.

The contribution of PGs to total ACh induced dilation was calculated as differences in the area under curves (AUC) for ACh induced dilation in absence and presence of indomethacin for individual groups. These data are shown in Figure 3.

In renal arteries, ACh induced dilation was not altered by lisinopril per se or by dietary sodium (data not shown). However, the combination of lisinopril and low sodium reduced the ACh induced dilation to approximately a third (Figure 2A). Incubation with indomethacin had no effect in CON and CON-LIS but partly restored vasodilation in LS-LIS (figure 3A), suggesting significant activity of contractile prostaglandins in the latter. In the mesenteric arteries, a similar reduction in ACh-induced dilatation during LS-LIS was observed, albeit to a lesser extent (Figure 2B). Incubation with indomethacin significantly further reduced the response in the LS-LIS group while leaving ACh-induced dilation in CON and CON-LIS unaffected (Figure 3B). These findings suggest a more prominent role for dilatory prostaglandins in mesenteric as compared to renal arteries during LS-LIS.

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Discussion

In accord with our hypothesis, we found that dietary sodium restriction – along with an increased blood pressure response - modifies the vascular effects of maintenance treatment with ACEi in resistance vessels. These effects were not uniform across the vascular bed. Baseline vessel characteristics were modified by ACEi in renal vessels, without a further change during the combination with low sodium, whereas in mesenteric vessels, ACEi as such had no effect, but the combination with low sodium led to increased myogenic tone and alpha-adrenergic responsiveness. Endothelial function was modified by the combination ACEi with low sodium as compared to ACEi in both vascular beds, with reduced endothelium-dependent vasodilation. In mesenteric vessels, this was associated with an increased role of vasodilator prostaglandins, whereas in renal vessels this was associated with an increase in vasoconstrictor prostaglandins. Whether these effects are cause or consequence of the enhanced response to ACEi cannot be derived from our data.

In mesenteric arteries, lisinopril per se did not have an effect on baseline vascular parameters. However, additional sodium restriction enhanced the propensity to increased vasoconstriction, a situation more likely to be a counter action than the cause of an enhanced blood pressure reduction.

The lisinopril per se induced changes in renal arteries are in line with increased renal blood flow found in experimental (Numabe et al., 1994) and human studies (Heeg et al., 1989; Navis et al., 1987). Also, vascular remodeling with increased vessel dimensions in response to long-term increase in flow following ACE inhibitor therapy seems in line with earlier studies (Reddi and Bollineni, 2001; Gibbons and Dzau, 1994). However, it should be noted that the in vivo effects of ACE inhibition on renal hemodynamics also involve effects on post-glomerular vessels (Heeg et al., 1989), leading to an altered balance of pre- and post-glomerular resistance. The resulting reduction in glomerular pressure likely contributes to the long-term renoprotective effects of ACE inhibition, in addition to the effects of lower systemic blood pressure (Heeg et al., 1989; Sanchez et al., 1991). In the present study, the effect of lisinopril on pre-glomerular renal arteries was not modified by dietary sodium restriction, implying that an enhanced response to ACE inhibition is not due to change in baseline vascular

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morphology or function but probably due to the enhanced blood pressure reduction. Thus, whereas mesenteric arteries are considered resistance vessels regulating blood pressure (Luscher et al., 1992) and become constricted during reduced blood pressure, the renal vessels ensure renal blood flow and remain dilated. Therefore, the effect of additional sodium on baseline vascular beds reveals the heterogeneity of their function rather than explaining enhanced therapy response.

Endothelium dependent dilation during maintenance ACE inhibition per se did neither improve nor attenuate in the present study. This may seem at variance with many studies reporting endothelial function during ACE inhibition in cardiovascular disease (Nunes et al., 2000; Hutri-Kahonen et al., 1997; Atkinson, 1995; Enseleit et al., 2001; Mancini, 2000). However, less is known about the effect of maintenance treatment with ACE inhibitors on apparently normal endothelial function in healthy conditions. In aortic rings of normal Wistar rats kept on a regular sodium diet, maximal dilation to ACh was increased from 70% in untreated rats to 90% after 6-weeks ramipril treatment (Berkenboom et al., 1995). In the present study, however, we studied small mesenteric resistance arteries and renal arteries which already showed near 100% relaxation to ACh; i.e. unlike the aorta there may not be much to be gained by ACE inhibition. Atkinson *et al* .found improved maximal relaxation to ACh in mesenteric arteries of normal WAG/Rij rats after ACE inhibitor treatment. However, the untreated rats in their study developed a time-dependent decrease in maximal ACh induced dilation in mesenteric artery, suggesting an improvement of ACh induced relaxation due to prevention of age-induced endothelial dysfunction (Atkinson, 1995; Atkinson et al., 1994). In our three weeks treatment compared to the treatment of several months of Atkinson et al., reduction of age-induced dysfunction due to ACE inhibition could not be expected.

In combination with low sodium, ACE inhibitor therapy reduced ACh induced relaxation both in small renal and mesenteric arteries. One other rat study also reported impairment of apparently normal endothelial function in renal arteries after chronic therapy (Barton et al., 2000). After treatment with the ET_A receptor antagonist LU135252, the relaxation of renal arteries to ACh was reduced in salt-treated salt-resistant Dahl rats. Interestingly, COX-inhibition with indomethacin acutely normalized this impairment. Evidence from studies with spontaneously hypertensive rats using indomethacin and PGH_2/TXA_2 receptor blockers (e.g. SQ 29,548)

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indicate that endothelium-derived PGH_2 and TXA_2 are contractile factors in intrarenal arteries that may underlie impaired relaxation to ACh (Dai et al., 1992; Fu-Xiang et al., 1992). Numerous studies have addressed the role of prostaglandins during changes in dietary sodium (Hocherl et al., 2002), but the impact on small vessels is less well known. In the present study, indomethacin also partially restored ACh induced relaxation of renal arteries of lisinopril treated rats during low sodium. Thus, our findings support involvement of COX-derived vasoconstrictive PGs – such as PGH_2 and TXA_2 - in development of decreased ACh-induced dilation in renal arteries during LS-LIS. The exact identity of the PG involved however, cannot be determined from these data as we did not test specific PG-modulators.

Relaxation to ACh in mesenteric arteries of lisinopril treated rats during LS was also decreased, but in contrast to renal arteries, this occurred despite an apparent enhanced contribution of dilative prostaglandins. Hence, the effect of ACEi under LS on endothelium-derived prostaglandins seems to be differentially altered in the two artery types, with an increase in constrictive prostaglandins in renal, and an increase in dilative prostaglandins in mesenteric arteries. One way to explain this apparent discrepancy may be a differential involvement of specific COX-isoforms in both vascular beds. In recent years, two different COXs have been described (Smith et al., 1996). Of these, COX-1 is considered the constitutive isoform as it is predominantly expressed at constant levels. COX-2 is considered the inducible isoform, as its expression can be rapidly induced in cells involved in inflammation, including vascular endothelial cells. Interestingly, PGs are produced by COX-2 in much larger amounts compared with COX-1, which led to the hypothesis of the existence of “good” versus “bad” PGs. In this concept, COX-1 generates “good” PGs for physiological “house-keeping functions”, including regulation of renal blood flow, while COX-2 forms the “bad” PGs involved in inflammatory reactions and responsible for inflammatory signs like capillary edema and vasodilation (Parente and Perretti, 2003). However, the terms constitutive and inducible have been noted to be too strict to denote regulation of COX-1 and -2, and both COX-1 and COX-2 are apparently involved in physiological as well as pathophysiological processes (Katori and Majima, 2000; Vane et al., 1998). This raises the possibility that in our study COX-1 and -2 expression and/or function in the renal versus mesenteric arterial bed was differentially affected after ACEi during LS, resulting in opposite production of PGs after endothelial stimulation with ACh. Interestingly, exposure of the mesenteric

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vascular bed to indomethacin, SC-560 (selective inhibitor of COX-1), or NS-398 (selective inhibitor of COX-2) was reported to reverse the hyporeactivity to noradrenaline and the increased vasodilatation to ACh in portal hypertensive rats, with NS-398 being more potent than the two other inhibitors (Potenza et al., 2002). Such findings indicate that endothelial COX-1 and -2 may also differentially affect vascular reactivity within one vessel type (i.e. mesenteric) under certain conditions. It would be of interest therefore, to study the effects of low sodium during ACE inhibition employing specific inhibitors of COX-1 and -2, in combination with inhibitors of down-stream synthases and/or PG-receptor antagonists.

The impact of our findings on the target organ protection in disease conditions also remains to be studied. The effect of adding sodium restriction to ACEi on intermediate parameters can be classified as favorable – with further reduction of blood pressure and proteinuria. As to the vascular effects observed here, it is doubtful whether these are favorable, or should – by contrast – be considered as an unwanted side effect that limits the eventual therapeutic benefit of the enhanced effects on the blood pressure (and/or proteinuria) on outcome in terms of target organ protection. A prior study from our group provides support for the latter assumption. In experimental nephrotic syndrome (Wapstra et al., 1996) low sodium potentiated the responses to ACEi of blood pressure and proteinuria, as well as renal outcome in terms of end-organ damage (focal sclerosis). However, the improvement in end-organ-damage was considerably less than would have been expected from the improvement in blood pressure and proteinuria. If our present data implicate, that the enhanced efficacy of ACEi is accompanied by possibly unwanted vascular effects, this example illustrates that it would be unwise to discard low sodium as an adjunct to ACE inhibition, as still the overall outcome is better than with ACEi alone. Rather, our findings provide a rationale to design additional treatment strategies, to preserve the potentiated treatment effect while at the same time preventing possibly unfavorable vascular side effects. Considering the role of prostaglandins in the altered endothelial function, the combination with maintenance treatment with COX-inhibition would be of interest. However, the heterogeneity of the involvement of prostaglandins across the vascular bed should be specifically considered!

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In conclusion, the combination of low sodium with ACE inhibition results in distinct vascular effects, along with an enhanced blood pressure response in healthy animals. It is uncertain from our data whether the vascular effects are cause or consequence from the enhanced blood pressure response. Endothelium-derived vasodilation was reduced, which raises the possibility that the vascular effects are unfavorable in terms of long term organ protection. Further studies should explore the impact of these vascular changes on long term outcome in disease models, and investigate the potential of these vascular changes as a target for additional intervention. This should not be taken to discard low sodium as an adjunct to ACE inhibition, but rather as a rationale for further studies addressing the mechanism-of-actions of our therapies.

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Footnotes

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Reprint requests to H. Buikema, Ph.D. Department of Clinical Pharmacology, University of Groningen; Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands; email: h.buikema@med.rug.nl

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Legends for figures

Figure 1. Effect of vasoactive prostaglandins on the PE induced precontraction.

Bars represent the contractile response to a single dose of $3x10^{-7}$ M phenylephrine (PE) in renal (A; top panel), and small mesenteric arteries (B: bottom panel) from normal rats chronically treated with lisinopril while on a control diet (CON-LIS, 2.0% NaCl), or on a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON). Contractions were generally reduced in presence (+) of indomethacin (right side) as compared to absence (-) of indomethacin (left side). Data are mean±SEM of n=8-10 observations in all cases.

* indicates $P<0.05$ for CON-LIS versus CON.

† indicates $P<0.05$ for CON-LIS versus LS-LIS

§ indicates $P<0.05$ for –indomethacin versus +indomethacin

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Figure 2. Endothelium-dependent and -independent dilation in renal and mesenteric arteries.

Full concentrations-response curves to and acetylcholine (ACh) and nitroprusside (SNP) in renal arteries (A; top panel) and small mesenteric arteries (B: bottom panel) from healthy control rats (CON), rats chronically treated with lisinopril on a control diet (CON-LIS, 2% NaCl) or a low-sodium diet (LS-LIS, 0.05% NaCl). Data are mean±SEM of n=8-10 observations in case of ACh-induced dilation, and n=4-5 in case of SNP-induced dilation.

* indicates $P<0.001$ for CON-LIS versus CON (repeated measures ANOVA)

† indicates $P<0.05$ for CON-LIS versus LS-LIS (repeated measures ANOVA)

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Figure 3. Effect of vasoactive prostaglandins on the endothelial dependent relaxation.

Bars represent ACh induced dilation, given as the area under curve (AUC) in arbitrary units, in the absence (-, left side) and presence (+) of indomethacin (right side) in small mesenteric arteries (A: upper pannel), and renal arteries (B: lower pannel) from normal rats treated with lisinopril while on a control diet (CON-LIS, 2.0% NaCl), or on a low-sodium diet (LS-LIS, 0.05% NaCl), as compared to non-treated rats on control diet (CON). Data are mean±SEM of n=8-10 observations in all cases.

* indicates $P<0.05$ for LIS versus CON.

† indicates $P<0.05$ for HS versus LS

§ indicates $P<0.05$ –indomethacin versus +indomethacin

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Tables

Table 1. Rat characteristics

	CON	CON-LIS	LS	LS-LIS
Urinary Sodium (mg/d)	3.3±0.3	4.9±0.4*	0.66±0.1†	0.54±0.1†
Body Weight (g)	398±10	398±11	398±12	312±5*†
Systolic Blood Pressure (mmHg)	140±4	125±4*	143±6	102±2*†
Plasma ACE-activity (His-Leu nM/ml/min)	75±7	30±2*	68±6	23±4*

Rat characteristics after treatment for tree weeks on a control diet (CON, 2.0% NaCl), a low sodium diet (LS, 0.05% NaCl), treated either with vehicle or the ACE inhibitor lisinopril (LIS, 75 mg/l drinking water). Data are mean±SEM of n=8-10 observations in all cases.

* indicates P<0.05 for CON-LIS versus CON or LS-LIS versus LS.

† indicates P<0.05 for LS versus CON and LS-LIS versus CON-LIS.

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Table 2. Vessel characteristics

	CON	CON-LIS	LS-LIS
- Renal Arteries -			
Diameter (μm)	296±8	331±12*	325±1*
Wall thickness (μm)	35±3	42±3	42±4
Wall-to-lumen ratio	0.12±0.01	0.13±0.01	0.13±0.02
Myogenic tone (%)	2±2	0.4±0.4	2±2
PE-induced tone (%)	35±4	24±3*	26±4*
- Mesenteric Arteries -			
Diameter (μm)	339±6	343±6	336±12
Wall thickness (μm)	41±2	43±3	36±2
Wall-to-lumen ratio	0.12±0.01	0.13±0.01	0.11±0.01
Myogenic tone (%)	0.2±0.2	0.5±0.3	24±1*†
PE-induced tone (%)	4±2	5±4	46±3*†

Rat vessel characteristics after treatment for tree weeks on a control diet (CON, 2.0% NaCl), a low sodium diet (LS, 0.05% NaCl), treated either with vehicle or the ACE inhibitor lisinopril (LIS, 75 mg/l drinking water). Myogenic and PE (phenylephrine, $3x10^{-7}$ mol/L)-inducd tone are expressed as % constriction from baseline. Data are mean±SEM of n=8-10 observations in all cases.

* indicates $P<0.05$ versus CON.

† indicates $P<0.05$ for LS-LIS versus CON-LIS.

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Figure 1

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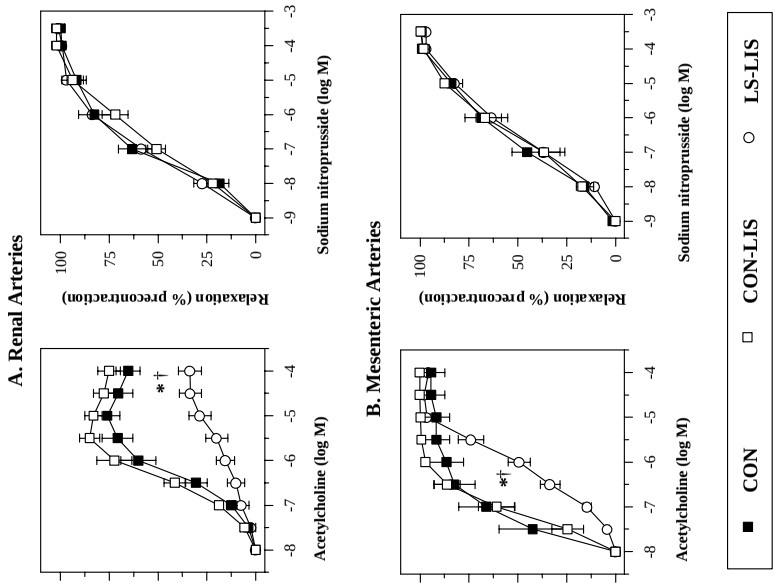

Figure 2

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Figure 3

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