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Enalapril treatment restores the decreased proximal tubule reabsorption in response to acute volume expansion in diabetic rats

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Abstract

The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure, fluid and electrolyte homeostasis. The RAS is activated and renal interstitial hydrostatic pressure (RIHP) is decreased in diabetic rats. The objective of this study was to evaluate the roles of proximal tubule reabsorption and RAS in the decreased RIHP and blunted natriuretic and diuretic responses to acute saline volume expansion (VE) in diabetic rats. Enalapril was utilized to inhibit angiotensin II (AII) formation. Diabetes mellitus (DM) was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ, 65 mg/kg). RIHP was measured by a polyethylene (PE) matrix that was chronically implanted in the left kidney. Fractional excretion of phosphate (FE_{Pi}) and fractional excretion of lithium (FE_{Li}) were used as indexes for proximal tubule reabsorption. VE significantly increased both FE_{Li} and FE_{Pi} in all groups of rats studied. However, the increase in FE_{Li} ($\Delta FE_{Li}=17.26\pm3.83\%$) and FE_{Pi} ($\Delta FE_{Pi}=7.38\pm2.37\%$) in diabetic rats (DC, $n=12$) were significantly lower as compared with those in nondiabetic control rats (NC, $n=8$; $\Delta FE_{Li}=32.15\pm4.71\%$ and $\Delta FE_{Pi}=20.62\pm3.27\%$). The blunted increases in FE_{Li} and FE_{Pi} were associated with an attenuated increase in RIHP ($\Delta RIHP$) in DC (1.8 ± 0.4 mmHg) compared with NC rats (4.3 ± 0.3 mmHg). Enalapril treatment (25 mg/kg/day in drinking water) had no effect on nondiabetic rats (NE, $n=8$) as compared with untreated NC rats, but significantly improved RIHP response ($\Delta RIHP$) to VE in diabetic rats (DE, $n=9$; 2.8 ± 0.5 mmHg). Both FE_{Li} and FE_{Pi} were restored by enalapril treatment in diabetic rats and no significant differences were found in ΔFE_{Li} and ΔFE_{Pi} between DE ($\Delta FE_{Li}=26.81\pm4.94\%$ and $\Delta FE_{Pi}=10.45\pm4.67\%$) and NC groups of rats in response to VE. These data suggest that the activated RAS and the decrease in RIHP may play an important role in the increased proximal tubule reabsorption, and the attenuated natriuretic and diuretic responses to acute volume expansion in diabetic rats.

Keywords

Diabetes mellitus; Renin-angiotensin system; Renal interstitial hydrostatic pressure; Volume expansion; Proximal tubule reabsorption

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Introduction

Diabetes mellitus (DM) is characterized by changes in fluid balance and blood volume homeostasis (Tang et al., 2004). Patients with type 1 diabetes have increased proximal tubular sodium reabsorption (Vervoort et al., 2005). Glomerular hyperfiltration is believed to play a role in the pathophysiology of diabetic nephropathy (Vervoort et al., 2005). It has been suggested that the increase in glomerular filtration rate (GFR) in DM might be caused by a primary increase in proximal tubular sodium reabsorption that ultimately leads to systemic volume expansion (Vervoort et al., 2005). Therefore, it is important to understand the mechanism(s) that lead to the increase in proximal tubular sodium reabsorption in DM as well as the response of proximal tubule reabsorption to stimuli such as volume expansion (VE).

The natriuretic and diuretic responses to VE are significantly attenuated in diabetic rats as compared to those of control nondiabetic rats (Tang et al., 2004; Patel and Carmines, 2001; Patel and Zhang, 1989, 1994; Phillips and Molitch, 2002). The exact cause of these attenuated natriuretic and diuretic responses to VE is not known; however, an increase in renal sympathetic nerve activity has been suggested to play an important role in the blunted natriuretic and diuretic responses to VE in diabetic rats (Patel and Zhang, 1989, 1994). Insulin treatment has been shown to partly reverse the increased renal sympathetic nerve activity and restore the renal excretory responses to VE in diabetic rats (Patel and Zhang, 1989, 1994; Phillips and Molitch, 2002). Restoration of blood glucose concentration levels to normal by immediate or delayed insulin treatment results in the normalization of basal renal interstitial hydrostatic pressure (RIHP) and RIHP response to VE; however, the increase in fractional excretion of sodium (ΔFE_{Na}), and the reduction in proximal tubule reabsorption responses (as evaluated by ΔFE_{Li} and ΔFE_{Pi}) are restored by immediate initiation of insulin treatment, but not by delayed insulin treatment in diabetic rats (Tang et al., 2004). Although these studies demonstrate a reversal of the increased renal sympathetic nerve activity by insulin treatment in diabetic rats (Patel and Zhang, 1989, 1994; Phillips and Molitch, 2002) and restoration of the reduction in proximal tubule reabsorption responses by immediate initiation of insulin treatment (Tang et al., 2004); however, the mechanism(s) that are responsible for the attenuated natriuretic, diuretic, and proximal tubule responses to VE in diabetic rats remains unclear.

The RAS plays an important role in controlling renal hemodynamics and sodium excretion and, therefore, regulation of body fluid volumes and blood pressure. Although plasma RAS is generally suppressed in DM (De Chatel et al., 1977; Feldt-Rasmussen et al., 1987). Pharmacological blockade of RAS, by an angiotensin converting enzyme inhibitor (ACEI) or by angiotensin II (AII) type I receptor (AT1) blockers, has renoprotective effects that are independent of change in blood pressure. These findings suggest that RAS might be an important mediator in the mechanisms that ultimately lead to renal pathogenesis during DM (Abbott and Bakris, 2002; Burns, 2000; Taniwaki et al., 2003). Therefore, the objectives of this study were to determine the cardiovascular and renal alterations during 4 wks of STZ-induced DM, and establish the relationship among RIHP, sodium and water excretion, and reabsorption by the proximal tubule. Furthermore, the role of RAS in these cardiovascular and renal alterations was evaluated by utilizing enalapril, an ACEI, to inhibit AII formation in diabetic rats.

Materials and methods

All rats in these studies were male Sprague-Dawley (SD) rats purchased from Harlan Sprague Dawley (Indianapolis, IN) when they were 11–12 wks old, and were housed in a temperature and humidity controlled animal facility with a 12:12 hr-light-dark cycle. All rats were fed Teklad Global 18% Protein Rodent Diet (Harlan, Madison, WI) and had free access to water. All protocols in this study were conducted in accordance with the National Institutes of Health

guidelines and were approved by the Institutional Animal Care and Use Committee of Eastern Virginia Medical School.

Polyethylene (PE) matrix implantation

The implantation procedure of the PE matrix has been previously described (Khraibi and Knox, 1989). RIHP was measured directly and continuously via a PE matrix that was implanted in the left kidney of rats when they were 12–13 wks old and weighed 200–280 g.

Induction of DM

Diabetes was induced by an intraperitoneal (i.p.) injection of 65 mg/kg body weight (wt) of streptozotocin (STZ, Pharmacia & Upjohn Company, Kalamazoo, MI). STZ was diluted in 0.1 mol/l citrate buffer (pH = 4.0). Normal control rats were given an equivalent amount of citrate buffer. Diabetes was diagnosed two days after STZ injection by measuring the blood glucose (BG) concentration from the cut tip of the tail by the glucose oxidase technique using One Touch II blood glucose meter (Lifescan Inc., Milpitas, CA). Only rats that received STZ injection and had a BG concentration > 15 mmol/l were included in the diabetic groups of rats (Osicka et al., 2000).

Four groups of male SD rats were studied in these experiments.

Normal control group (NC, n=8)—These were normal control nondiabetic male SD rats that did not receive any treatment.

Diabetic group (DC, n=12)—These were diabetic male SD rats that did not receive insulin treatment.

Diabetic + enalapril treatment group (DE, n=9)—These were diabetic rats that received enalapril treatment (25 mg/kg/day in drinking water).

Normal control + enalapril treatment group (NE, n=8)—These were control nondiabetic rats that received enalapril treatment (25 mg/kg/day in drinking water).

Surgical procedure for VE experiments

The acute terminal experiments were performed 4 weeks after the confirmation of diabetes (4 weeks and 2 days after STZ injection) and at similar period for the nondiabetic groups. On the day of the acute experiment, rats were anesthetized with Inactin (100 mg/kg) and catheters were placed in the trachea (PE-240), and left jugular vein (PE-50) for intravenous infusion of 1.5 ml/100 g body wt/h of saline with 6 mM lithium chloride (LiCl) and 1.5 ml/100 g body wt/h of a solution of 3% inulin and 6.25% bovine albumin in saline (with 6 mM LiCl). A PE-50 catheter was implanted in the left carotid artery for mean arterial pressure (MAP) measurement and blood withdrawal. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. The rats were allowed 1 hour to recover after completion of the surgical procedures. Then a control period of 30 min was started. During the 30-min clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period approximately 0.5 ml of blood was withdrawn from the left carotid artery for plasma electrolytes, lithium, and inulin measurements. At this time VE (5% body wt saline in 30 min) was started. Urine was collected for 20 min starting 10 min after the initiation of VE. Again, during the second clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period, about 0.5 ml of blood was withdrawn from the left carotid artery for plasma electrolytes, lithium, and inulin measurements. All rats were killed by air embolism at the end of the experiment while still under deep Inactin anesthesia, and both kidneys were excised and

weighed. This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Measurements in plasma and urine

GFR was calculated from the clearance of inulin, and inulin concentrations were measured by the anthrone method (Führ et al., 1955). Sodium and lithium concentrations in plasma and urine were measured using flame photometry (Instrumentation Laboratory model 943, Lexington, MA). Phosphate concentrations in plasma and urine were measured according to the method of Chen et al. (Chen et al. 1956).

Statistical analyses

Statistical analyses were performed using SPSS 10.0 statistical software (SPSS Inc, Chicago, IL). The data were analyzed with standard paired Student's *t* tests for comparisons between the first and second clearance periods in the same group or with a one-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni correction for multiple group comparisons at equivalent periods. All data are means $\pm$ SE, and $P < 0.05$ was accepted as a statistically significant difference.

Results

The results of these studies are shown in Tables 1 and 2, and Figs. 1 to 3.

Characteristics of nondiabetic and diabetic rats

As shown in Table 1, on the day of the acute terminal experiments, diabetic rats (groups DC and DE) had significantly higher BG concentration than nondiabetic rats (groups NC and NE). Although all groups of rats had similar body wt on the day of the single injection of STZ or vehicle (data not shown), diabetic rats had significantly lower body wt, significantly higher kidney wt, and significantly higher kidney wt/body wt ratio as compared with nondiabetic rats (Table 1). Systemic arterial pressure (SAP) as measured by the tail-cuff method started to increase on day 15 of diabetes, while heart rate (HR) decreased starting on day 3 of diabetes in diabetic rats as compared with nondiabetic control rats (SAP and HR were measured every three days; data not shown). Enalapril treatment (25 mg/kg/day in drinking water) had no effect on the above measurements in nondiabetic rats (NE group) as compared to nondiabetic untreated control rats (NC group, Table 1). In diabetic rats, although enalapril treatment (DE group) had no effect on BG concentrations and body wt as compared to untreated diabetic rats (DC group); it had partial but significant improvement on the changes in kidney wt and HR, and restored SAP (DE vs. NC groups, Table 1).

Effect of diabetes and enalapril treatment on renal proximal tubular reabsorption

Fractional excretion of phosphate (FE_{P_i}) and fractional excretion of lithium (FE_{Li}) were measured to evaluate the effects of diabetes and enalapril treatment on renal proximal tubular reabsorption (Fig. 1 and Table 2). VE significantly increased both FE_{Li} and FE_{P_i} in all groups of rats studied (Fig. 1), suggesting a significant reduction in proximal tubular reabsorption. However, the increase in FE_{Li} ($\Delta FE_{Li} = 17.26 \pm 3.83\%$) and FE_{P_i} ($\Delta FE_{P_i} = 7.38 \pm 2.37\%$) in diabetic rats (DC group) were significantly lower as compared with those in nondiabetic control rats (NC group; $\Delta FE_{Li} = 32.15 \pm 4.71\%$ and $\Delta FE_{P_i} = 20.62 \pm 3.27\%$). Enalapril treatment had no effect on nondiabetic rats (NE group) as compared with untreated NC rats, but restored both ΔFE_{Li} and ΔFE_{P_i} in diabetic rats (DE group). No significant differences were found in ΔFE_{Li} and ΔFE_{P_i} between DE ($\Delta FE_{Li} = 26.81 \pm 4.94\%$ and $\Delta FE_{P_i} = 10.45 \pm 4.67\%$) and NC ($\Delta FE_{Li} = 32.15 \pm 4.71\%$ and $\Delta FE_{P_i} = 20.62 \pm 3.27\%$) groups of rats in response to VE.

Enalapril treatment partially prevents the decrease in RIHP and restores the natriuretic response to VE in diabetic rats

As shown in Table 2 and Figure 2, compared with NC rats, DC rats had a significantly lower basal RIHP (3.0 ± 0.2 mmHg for DC vs. 6.8 ± 0.4 mmHg for NC rats) and an attenuated increase in RIHP in response to VE (Δ RIHP, 1.8 ± 0.4 mmHg for DC vs. 4.3 ± 0.3 mmHg for NC rats). Enalapril treatment had no effect on the above parameters in normal rats, but partially restored basal RIHP (5.1 ± 0.7 mmHg) and Δ RIHP (2.8 ± 0.5 mmHg) in diabetic rats (DE group, Table 2 and Fig. 2). No significant differences in GFR were found between nondiabetic and diabetic rats (Table 2). Consistent with the RIHP data, the natriuretic responses to VE (Δ FE_{Na} and Δ U_{Na}V) were significantly lower in DC ($3.78 \pm 0.54\%$ for Δ FE_{Na} and 7.11 ± 0.90 μ Eq/min/g k.w. for Δ U_{Na}V) compared with NC ($7.11 \pm 1.65\%$ for Δ FE_{Na} and 11.38 ± 1.02 μ Eq/min/g k.w. for Δ U_{Na}V) groups of rats (Table 2 and Fig. 3). Enalapril treatment restored the natriuretic response to VE in diabetic rats ($4.74 \pm 0.79\%$ for Δ FE_{Na} and 9.99 ± 2.21 μ Eq/min/g k.w. for Δ U_{Na}V) since there was no significant differences in Δ FE_{Na} and Δ U_{Na}V between DE and NC groups of rats (Fig. 3). However, FE_{Na} remained significantly lower in DE group as compared to NC group of rats during both control and VE periods (Table 2).

Discussion

In DM there are changes in fluid balance, cardiovascular hemodynamics, and blood volume homeostasis (Tang et al., 2004). Also, DM is characterized by glomerular hyperfiltration, an activated RAS, a decrease in RIHP, an increase in proximal tubular sodium reabsorption and systemic volume expansion (Vervoort et al., 2005). The natriuretic and diuretic responses to VE are significantly attenuated in diabetic rats as compared to those of control nondiabetic rats (Tang et al., 2004; Patel and Carmines, 2001; Patel and Zhang, 1989, 1994; Phillips and Molitch, 2002). With VE, there is a significant decrease in proximal tubule reabsorption in control nondiabetic rats, but this decrease in proximal tubule reabsorption is blunted in diabetic rats resulting in attenuated natriuretic and diuretic responses to VE in these rats (Tang et al., 2004). In the present study, the roles of RAS and proximal tubule reabsorption in the decreased RIHP and the blunted natriuretic and diuretic responses to VE in diabetic rats were determined.

The RAS plays an important role in the regulation of blood pressure, sodium and fluid regulation. The intrarenal RAS is activated in DM. Recently, it has been suggested that intrarenal RAS, specifically within glomeruli and proximal tubules, may be activated by hyperglycemia (Burns, 2000). This activation can lead to stimulation of local AII production, which may exert feedback inhibition on systemic RAS. Renal responsiveness to angiotensin is enhanced in diabetic rats (Kennefick et al., 1996). AII exerts most of its actions by binding AT1 receptors, which are abundantly present in cells of the glomeruli, renal tubules and vasculature, and renal interstitium. AII causes renal vasoconstriction thus reducing renal blood flow, and directly or indirectly increases sodium reabsorption. AII vasoconstricts both afferent and efferent arterioles, but has a preferential effect on the efferent arterioles (Yuan et al., 1990). Therefore, the combined effects of AII contribute to the increased glomerular capillary hydrostatic pressure, and increased tubular sodium reabsorption in DM. The RAS is activated and RIHP is decreased during DM. Therefore, the objective of this study was to evaluate the roles of RAS and proximal tubule reabsorption in the decreased RIHP and blunted natriuretic and diuretic responses to VE in diabetic rats. Enalapril, an ACEI, was utilized to inhibit AII formation.

The results of this study, and those of others (Tang et al., 2004; Patel and Carmines, 2001), showed that basal RIHP and the increase in RIHP that is associated with natriuresis and diuresis responses to VE were reduced in STZ-induced diabetic rats (Table 2 and Figs. 2 and 3). Since phosphate and lithium are almost exclusively reabsorbed in the proximal tubule; therefore, FE_{Pi} and FE_{Li} were used as indexes for proximal tubule reabsorption (Yu and Khraibi, 2005).

Volume expansion significantly increased both FE_{Li} and FE_{Pi} in all groups of rats studied (Fig. 1), indicating a significant reduction in proximal tubule reabsorption in response to VE. However, ΔFE_{Li} and ΔFE_{Pi} in untreated diabetic rats (DC group) were significantly lower as compared with those in nondiabetic control rats (NC group). The blunted ΔFE_{Li} and ΔFE_{Pi} were associated with an attenuated increase in RIHP ($\Delta RIHP$) in DC group as compared with NC group of rats (Fig. 2). In this study, the activated RAS that is associated with DM was inhibited by treatment with the ACEI enalapril in the drinking water of diabetic rats (DE group). Enalapril treatment had no effect on $\Delta RIHP$ of nondiabetic rats (NE group) as compared with untreated nondiabetic control rats (NC group), but significantly improved the increase in RIHP ($\Delta RIHP$) to VE in diabetic rats (DE group; Table 2 and Fig. 2). As shown in Figure 1, both ΔFE_{Li} and ΔFE_{Pi} were restored by enalapril treatment in diabetic rats, and no significant differences were found in ΔFE_{Li} and ΔFE_{Pi} between DE and NC groups of rats in response to VE.

AII increases renal tubular sodium reabsorption partly by increasing the activity of $Na^+-K^+-ATPase$ and Na^+-H^+ exchanger type 3 (NHE-3) in the proximal tubule (Shah and Hussain, 2006; Hakam and Hussain, 2006; Turban et al., 2006). Therefore, it is reasonable to assume that the administration of an ACEI such as enalapril would reduce proximal tubular sodium reabsorption, at least in part by reducing the activity of $Na^+-K^+-ATPase$ and NHE-3, and cause natriuresis and diuresis. Elevations in RIHP that are mediated by volume expansion decrease sodium reabsorption by the proximal tubule and loop of Henle (Granger, 1986). The exact mechanism(s) of how changes in RIHP result in alterations in sodium reabsorption and excretion is not yet known. However, several possible mechanisms to explain these relationships have been suggested by many investigators. These mechanisms include an increase in the back-leak of fluid and solutes into the proximal tubule and/or increases in medullary blood flow and ultimately in medullary washout as a result of increases in RIHP. These possible renal changes mediated by increases in RIHP would ultimately result in a decrease in sodium reabsorption and an increase in sodium excretion (Patel and Carmines, 2001; Khraibi and Knox, 1989; Khraibi et al., 2002). In the present study, basal RIHP, increases in RIHP, the increase in FE_{Li} and FE_{Pi} , and the natriuretic and diuretic responses to VE were diminished in diabetic rats as compared to nondiabetic control rats (DC vs. NC groups; Table 2, Figs. 1–3). Enalapril treatment significantly improved $\Delta RIHP$, and restored ΔFE_{Li} and ΔFE_{Pi} in response to VE in diabetic rats (DE group; Table 2 and Figs. 1 and 2).

Conclusion

The data in the present study suggest that the activated RAS and the decrease in RIHP may play an important role in the attenuated decrease in proximal tubule reabsorption and the blunted natriuretic and diuretic responses to acute volume expansion in diabetic rats.

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Figure 1. Fractional excretion of lithium (ΔFE_{Li} , A) and fractional excretion of phosphate (ΔFE_{Pi} , B) in response to volume expansion (VE) in nondiabetic control rats (NC, n=8); nondiabetic rats with enalapril treatment (NE, n=8); diabetic rats (DC, n=12); diabetic rats with enalapril treatment (DE, n=9). *Significant difference compared with nondiabetic control rats using a 1-way ANOVA, followed by a post hoc Bonferroni correction.

Figure 2.

Renal interstitial hydrostatic pressure (Δ RIHP) in response to volume expansion (VE) in nondiabetic control rats (NC, n=8); nondiabetic rats with enalapril treatment (NE, n=8); diabetic rats (DC, n=12); diabetic rats with enalapril treatment (DE, n=9). *Significant difference compared with nondiabetic control rats using a 1-way ANOVA, followed by a post hoc Bonferroni correction.

Figure 3. Urinary sodium excretion ($\Delta U_{Na}V$) and fractional excretion of sodium (ΔFE_{Na} , B) in response to volume expansion (VE) in nondiabetic control rats (NC, n=8); nondiabetic rats with enalapril treatment (NE, n=8); diabetic rats (DC, n=12); diabetic rats with enalapril treatment (DE, n=9). *Significant difference compared with nondiabetic control rats using a 1-way ANOVA, followed by a post hoc Bonferroni correction.

Table 1

Characteristics of nondiabetic and diabetic rats

	Blood Glucose (mmol/L)	Body wt (g)	Kidney wt (g)	Kidney wt/100 g body wt	SAP (mmHg)	HR (beats/min)
NC (n=8)	4.39 ± 0.25	390 ± 4	2.65 ± 0.04	0.68 ± 0.01	128 ± 2	361 ± 4
NE (n=8)	4.08 ± 0.20	393 ± 5	2.75 ± 0.03	0.70 ± 0.01	127 ± 1	361 ± 4
DC (n=8)	26.30 ± 0.34 [*]	348 ± 6 [*]	3.49 ± 0.10 [*]	1.01 ± 0.03 [*]	137 ± 4 ^{‡a}	302 ± 5 ^{‡a}
DE (n=9)	25.22 ± 0.98 [*]	346 ± 5	3.14 ± 0.06 [‡]	0.91 ± 0.02 [‡]	123 ± 1 [‡]	316 ± 3 [‡]

Values are means ± SE; n, number of rats. NC, nondiabetic control rats; NE, nondiabetic rats with enalapril treatment; DC, diabetic control rats; DE, diabetic rats with enalapril treatment; SAP, systolic arterial pressure; HR, heart rate.

^{*} Significant difference ($P < 0.05$) vs. NC rats.

[‡] Significant difference ($P < 0.05$) vs. DC rats.

^a n = 6 for measurements of SAP and HR.

Changes in renal responses to acute saline volume expansion (VE, 5% b.w. per 30 minutes) during DM and the effects of Enalapril treatment

Table 2

	NC (n=8)		NE (n=8)		DC (n=12)		DE (n=9)	
	Pre-VE	VE	pre-VE	VE	pre-VE	VE	pre-VE	VE
MAP (mmHg)	123 ± 2	110 ± 2 [*]	126 ± 3	111 ± 4 [*]	121 ± 4	104 ± 3 [*]	120 ± 2	103 ± 1 [*]
RIHP (mmHg)	6.8 ± 0.4	11.1 ± 0.4 [*]	7.0 ± 0.2	12.0 ± 0.4 [*]	3.0 ± 0.2 [†]	4.8 ± 0.5 [†]	5.1 ± 0.7 [†]	7.9 ± 1.1 [†]
GFR (ml/min/g k.w.)	1.31 ± 0.06	1.30 ± 0.16	1.28 ± 0.15	1.20 ± 0.08	1.44 ± 0.19	1.44 ± 0.19	1.31 ± 0.14	1.58 ± 0.23
U _{Na} V (μEq/min/g k.w.)	3.63 ± 0.53	15.01 ± 0.85 [*]	2.62 ± 0.16	15.61 ± 1.11 [*]	0.57 ± 0.07 [†]	7.68 ± 0.87 [†]	0.99 ± 0.20 [†]	10.98 ± 2.20 [*]
FE _{Na} (%)	2.05 ± 0.36	9.16 ± 1.60 [*]	1.60 ± 0.21	9.48 ± 1.10 [*]	0.32 ± 0.05 [†]	4.09 ± 0.55 [†]	0.55 ± 0.12 [†]	5.29 ± 0.85 [†]
FE _{Li} (%)	26.23 ± 2.68	58.39 ± 6.11 [*]	32.75 ± 5.39	61.43 ± 3.68 [*]	21.13 ± 4.31	38.39 ± 3.78 [†]	26.82 ± 4.99	53.63 ± 7.96 [*]
FE _{Na} (%)	9.26 ± 2.26	29.89 ± 5.21 [*]	4.88 ± 2.29	17.77 ± 3.65 [*]	1.85 ± 0.79 [†]	9.23 ± 3.02 [†]	4.91 ± 2.31	15.36 ± 6.68 [*]
V (μl/min/g k.w.)	20.98 ± 3.20	104.17 ± 4.31 [*]	16.80 ± 1.21	111.80 ± 7.57 [*]	16.81 ± 2.09	53.07 ± 5.10 [†]	17.73 ± 2.77	52.62 ± 5.33 [†]

Values are mean ± SE. n, number of rats. NC, nondiabetic control rats; NE, nondiabetic rats with enalapril treatment; DC, diabetic control rats; DE, diabetic rats with enalapril treatment; MAP, mean arterial pressure; RIHP, renal interstitial hydrostatic pressure; GFR, glomerular filtration rate; U_{Na}V, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; FE_{Li}, fractional excretion of lithium; FE_{Pi}, fractional excretion of phosphate; V, urine flow rate.

* Significant difference ($P < 0.05$) between pre-VE and VE periods.

[†] Significant difference ($P < 0.05$) vs. NC rats.