ONLINE SUPPLEMENT

Relationship between renin-angiotensin-aldosterone system and renal $K_{ir}5.1$ channels

Anna D. Manis*, Oleg Palygin*, Sherif Khedr, Vladislav Levchenko, Matthew R. Hodges, and Alexander Staruschenko

*equal contribution

Correspondence: Alexander Staruschenko, PhD. Department of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. Phone: (414) 955-8475; E-mail: staruschenko@mcw.edu

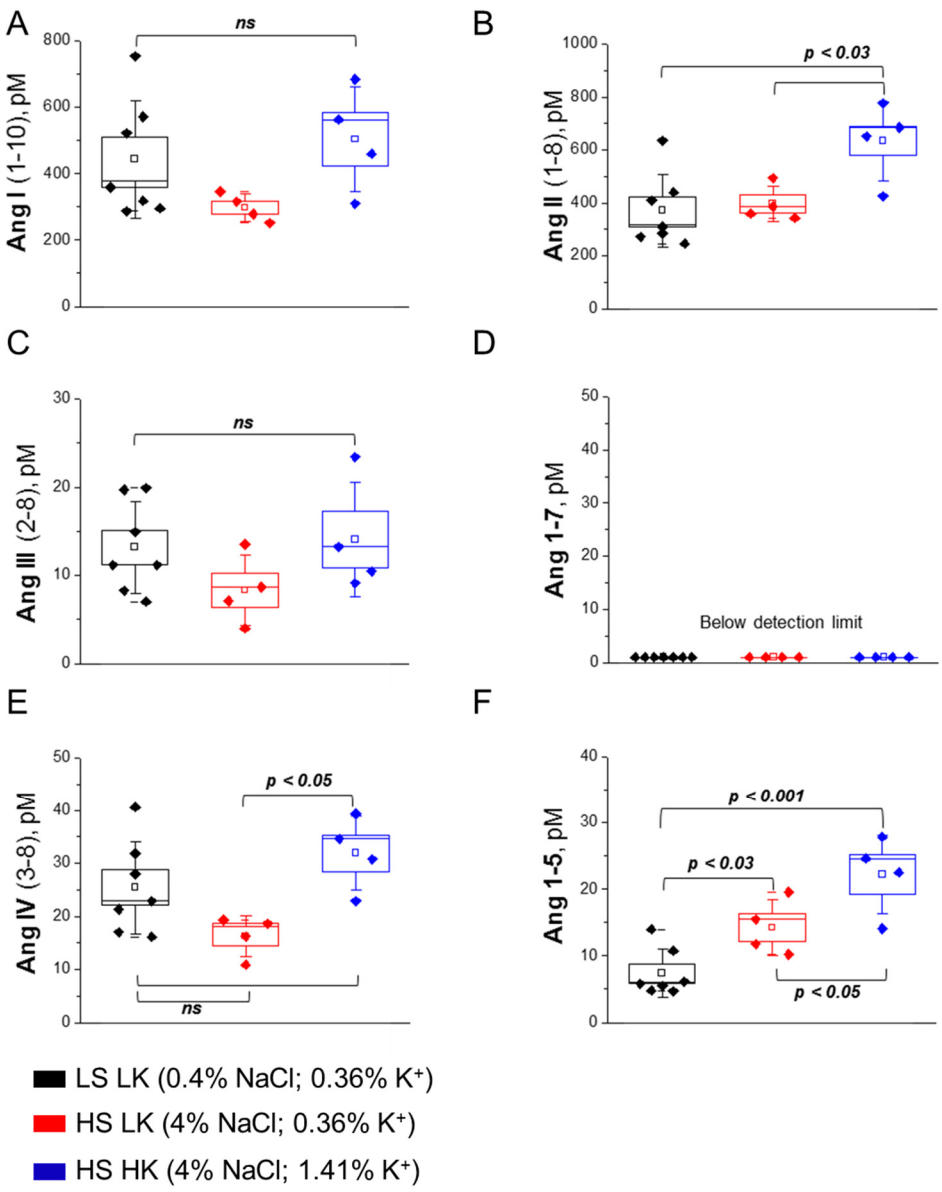

Figure S1. Quantification of equilibrium angiotensin metabolites in SS^{WT} rats (expanded view of Figure 1). Ang I (1-10) (**A**), Ang II (1-8) (**B**), Ang III (2-8) (**C**), Ang 1-7 (**D**), Ang IV (3-8) (**E**), and Ang 1-5 (**F**) were quantified from SS^{WT} plasma samples collected from rats fed one of 3 diets. In the boxplots, black data points denote rats fed a low salt, low potassium diet (LS LK, 0.4% NaCl and 0.36% K^+; N= 7 males); red indicates a high salt, low potassium diet (HS LK, 4% NaCl and 0.36% K^+; N=4 males); and blue indicates a high salt, high potassium diet (HS HK, 4% NaCl and 1.41% K^+; N=4 males). Measurements are shown in pM. *p*-values are given for each graph.

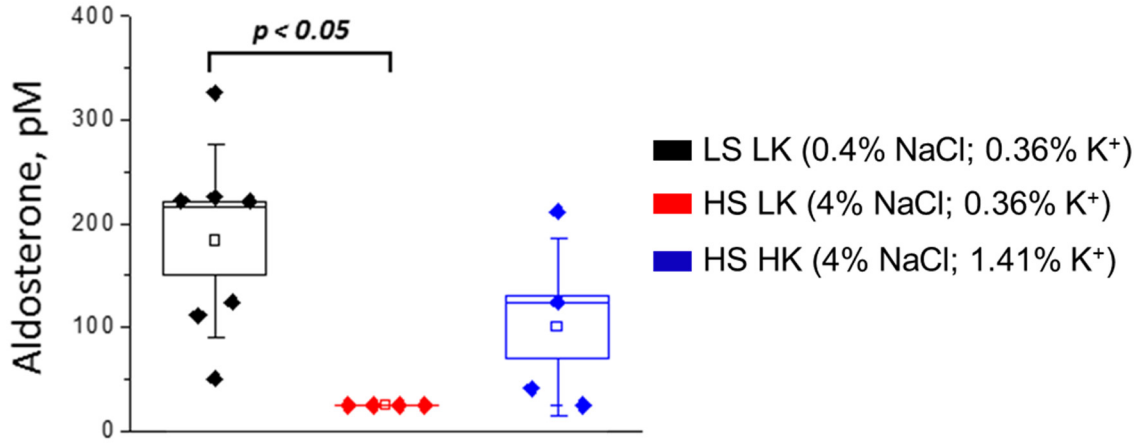

Figure S2. Quantification of plasma aldosterone in SS^{WT} rats (expanded view of Figure 2C). Equilibrium aldosterone was quantified from plasma samples collected from SS^{WT} rats fed one of 3 diets, indicated in the key. Groups consist of N=7, 4, and 4 male SS^{WT} rats fed LS LK, HS LK, and HS HK, respectively. Measurements are shown in pM and *p*-values are given.

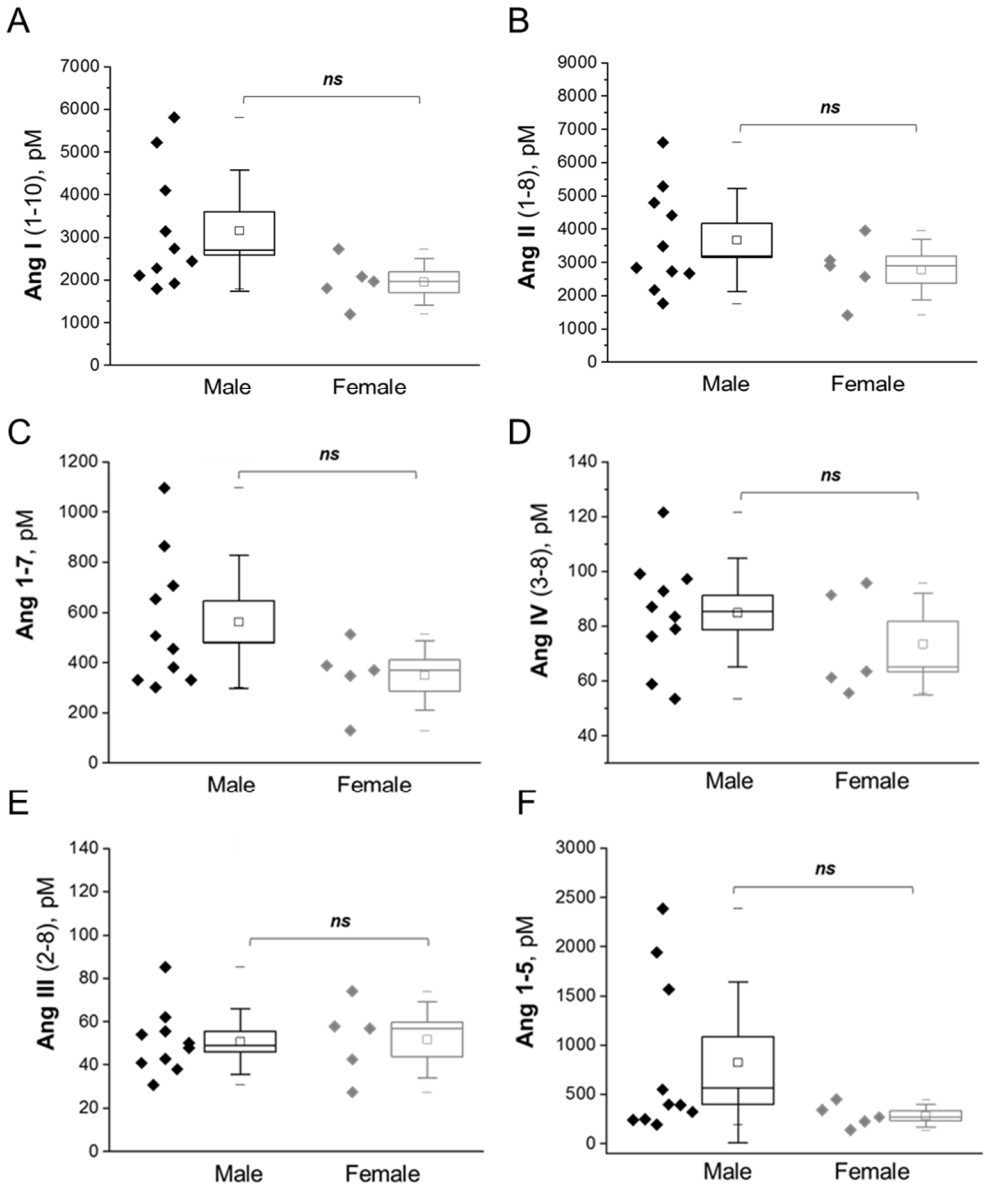

Figure S3. Sex differences in angiotensin metabolites. Equilibrium angiotensin metabolites were measured using mass spectrometry-based quantification of plasma samples from male (N = 10, black points) and female (N = 5, gray points) $SS^{Kcnj16-/-}$ rats on the standard low salt diet (0.4% NaCl). There were no significant differences in serum Ang I (**A**), Ang II (**B**), Ang III (**C**), Ang 1-7 (**D**), Ang IV (**E**), or Ang 1-5 (**F**) between male and female rats.

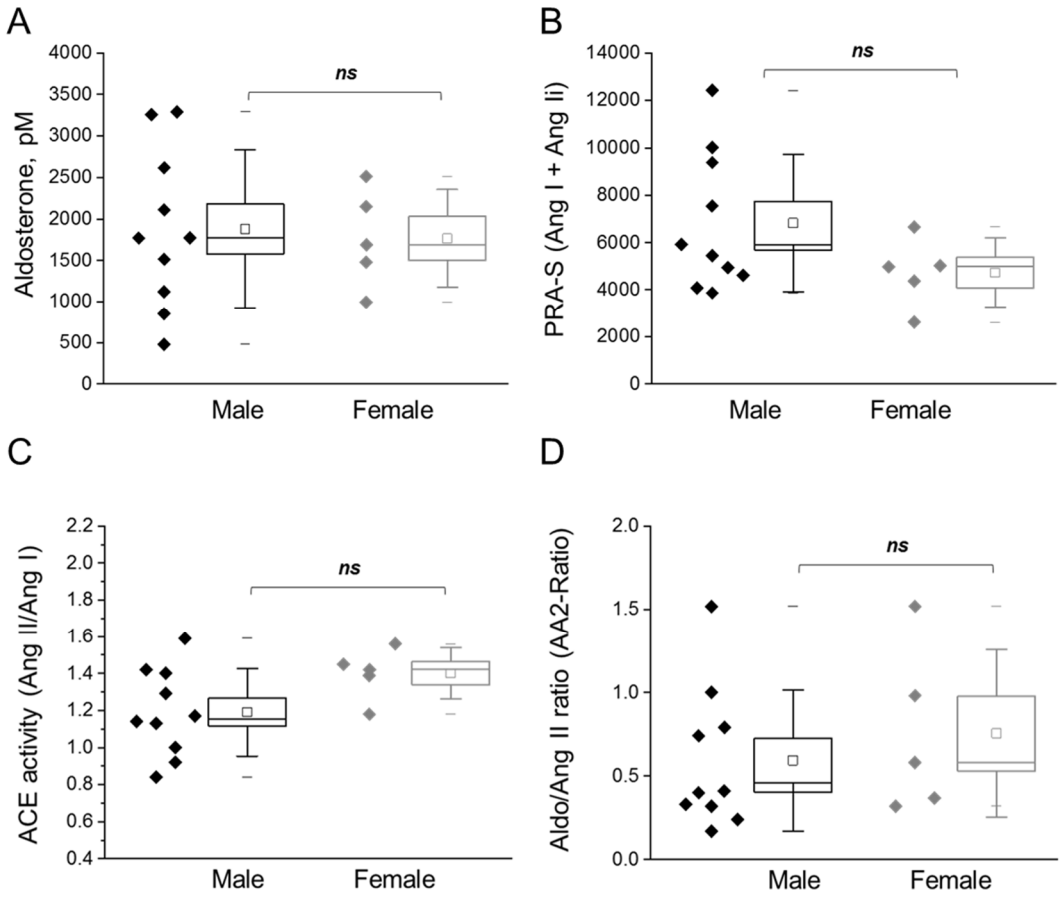

Figure S4. Sex differences in plasma aldosterone and surrogate RAAS measures. Equilibrium aldosterone and angiotensin metabolites were measured using mass spectrometry-based quantification of serum samples from male (N = 10, black points) and female (N = 5, gray points) $SS^{Kcnj16-/-}$ rats on a low salt diet (0.4% NaCl). (**A**) Aldosterone measurements represented in pM. (**B**) Plasma renin activity (PRA-S) is represented by the combined quantity of Ang I and Ang II in pM. (**C**) ACE activity indicated by the ratio of serum Ang II to Ang I. (**D**) The ratio of aldosterone to Ang II (AA2 ratio) to assess the adrenal response to Ang II. There were no significant differences between sexes in circulating aldosterone, PRA-S, ACE activity, or AA2 ratio.

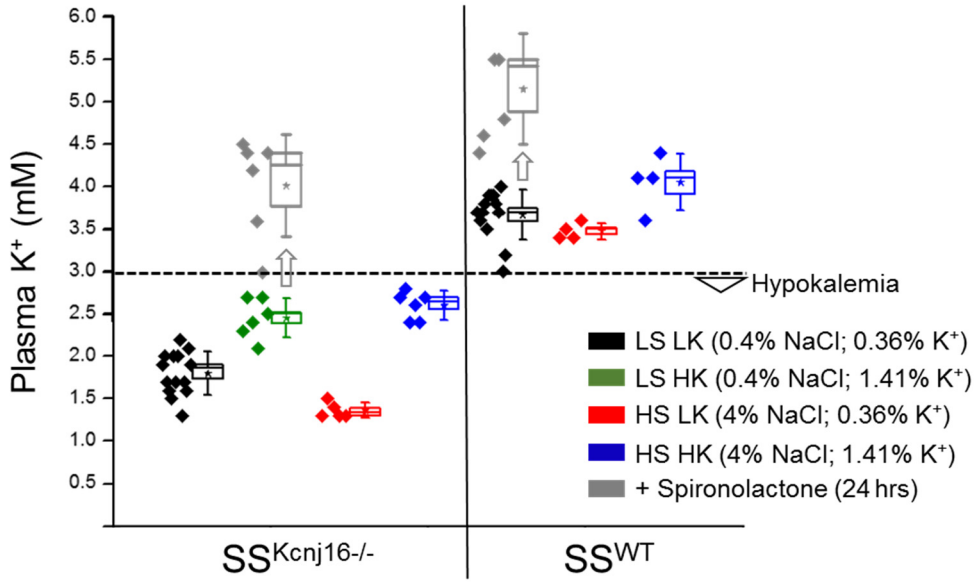

Figure S5. Plasma potassium measurements for experimental animals on each diet. All values below the dotted line at 3.0 mM are considered hypokalemic. Diet composition is indicated by color specified in the key. Data in gray represents increased plasma K^+ resulting from administration of spironolactone. Spironolactone data is from Table 1 and is depicted here for visual comparison.

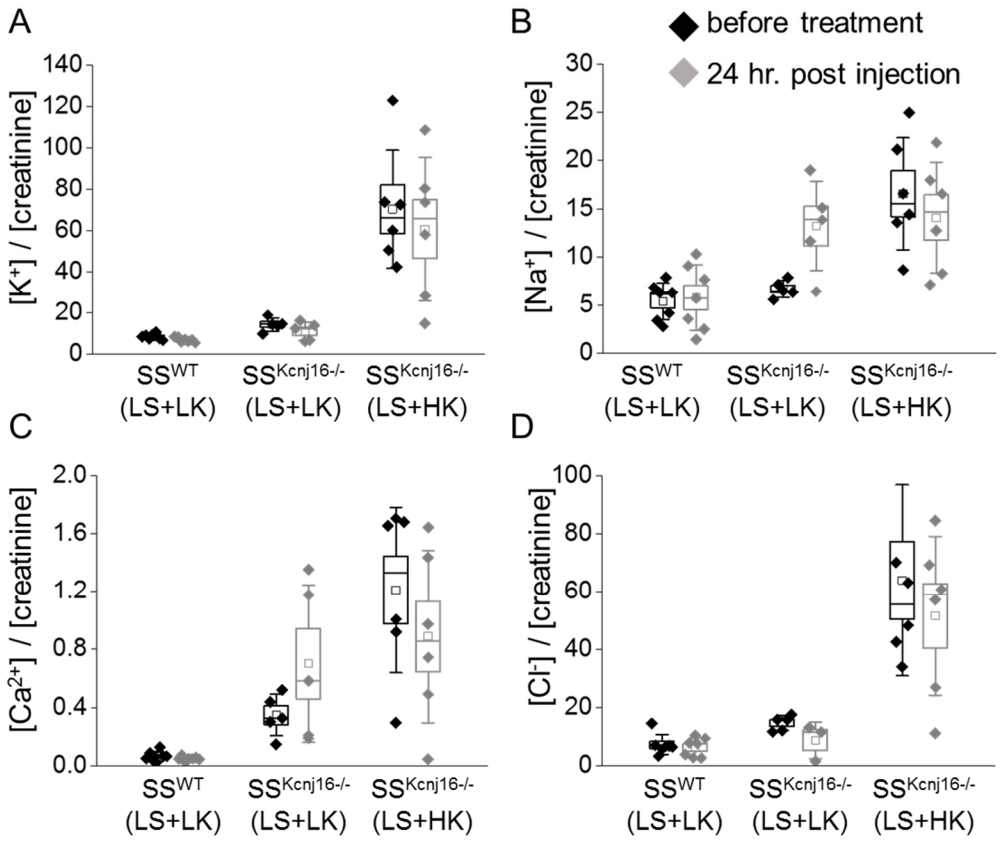

Figure S6. Changes in 24-hour urinary electrolyte excretion with spironolactone administration. Urine was collected and measured from SS^{WT} rats on a LS LK diet (0.4% NaCl and 0.36% K+; N=7 males), $SS^{Kcnj16-/-}$ rats on a LS LK diet (0.4% NaCl and 0.36% K+; N=5 males), and $SS^{Kcnj16-/-}$ rats on a LS HK diet (0.4% NaCl and 1.41% K+; N=6 males) before and 24 hours after spironolactone administration (50 mg/kg administered IP). Potassium (**A**), sodium (**B**), calcium (**C**), and chloride (**D**) measurements were normalized to the urinary concentration of creatinine (mmol/L).

	SS^{WT} (N=3)			$SS^{Kcnj16-/-}$ (N=4)		
ACEi Treatment:	-Drug (LS LK)	+Drug (LS LK)	+Drug (HS LK)	-Drug (LS LK)	+Drug (LS LK)	+Drug (HS LK)
K^+	7.1 ± 0.6	9.0 ± 1.3	12.9 ± 0.8	12.3 ± 1.4	14.6 ± 1.2	31.6 ± 2.3
Na^+	2.5 ± 1.4	8.3 ± 0.4	95.0 ± 5.1	6.5 ± 1.1	11.3 ± 0.6	100.8 ± 15.7
Ca^{2+}	0.06 ± 0.02	0.11 ± 0.03	0.53 ± 0.06	0.46 ± 0.04	0.35 ± 0.14	1.88 ± 0.99
Cl^-	4.9 ± 0.3	10.4 ± 1.3	102.8 ± 3.5	16.2 ± 2.3	15.9 ± 2.8	123.1 ± 8.4
	SS^{WT} (N=3)			$SS^{Kcnj16-/-}$ (N=4)		
ARB Treatment:	-Drug (LS LK)	+Drug (LS LK)	+Drug (HS LK)	-Drug (LS LK)	+Drug (LS LK)	+Drug (HS LK)
K^+	8.0 ± 0.7	10.3 ± 1.1	15.2 ± 1.4	10.0 ± 0.7	16.1 ± 2.8	35.8 ± 0.9
Na^+	1.7 ± 0.8	7.8 ± 1.0	82.2 ± 9.6	4.8 ± 1.1	10.8 ± 2.2	148.0 ± 14.6
Ca^{2+}	0.04 ± 0.01	0.09 ± 0.01	0.34 ± 0.06	0.39 ± 0.07	0.34 ± 0.04	3.70 ± 0.37
Cl^-	4.3 ± 0.7	11.8 ± 1.2	97.0 ± 10.6	14.1 ± 1.5	14.5 ± 4.9	143.0 ± 14.1

Table S1. Changes in urinary electrolyte excretion with daily ACEi (upper panel) or ARB (lower panel) administration. SS^{WT} and $SS^{Kcnj16-/-}$ rats were treated with daily IP injection of an ACEi (50 mg/kg Captopril; N=3 SS^{WT} males and N=4 $SS^{Kcnj16-/-}$ males) or ARB (30 mg/kg Losartan; N=3 SS^{WT} males and N=4 $SS^{Kcnj16-/-}$ males). Urine was collected for 24 hours and urinary excretion of potassium, sodium, calcium, and chloride were measured before any treatment (-Drug (LS LK)), after a week of daily treatment (+Drug (LS LK)), and 24 hours after switching the diet to high salt (+Drug (HS LK)). Electrolyte concentration measurements were normalized to the urinary concentration of creatinine (mmol/L).