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Primate Response to Angiotensin Infusion and High Sodium Intake Differ by Sodium Lithium Countertransport Phenotype

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Abstract

An increased level of sodium-lithium countertransport (SLC) activity has been associated with salt-sensitive hypertension. Previous findings have suggested that dysregulation of the renin-angiotensin-aldosterone system (RAAS) may be involved in the mechanism linking elevated SLC activity and hypertension. Therefore, baboons with different levels of SLC activity were given two diets differing in sodium content, with and without an angiotensin II (ANG II) infusion, to investigate the relationship between SLC activity, the RAAS, and physiological regulation by sodium. Although we anticipated that high SLC (HSLC) activity would be associated with inappropriate function of the RAAS and greater arterial pressure sensitivity to dietary sodium and ANG II and that low SLC (LSLC) activity would be associated with the least BP sensitivity, we found that the LSLC phenotype correlated with BP sensitivity similar to the HSLC phenotype, and the normal SLC (NSLC) phenotype showed the least BP sensitivity to dietary sodium and ANG II.

Keywords

Hypertension; SLC; Angiotensin; Baboon; Nonhuman Primate

INTRODUCTION

Hypertension is a complex trait determined by both genetic and environmental factors, such as dietary salt intake. Although salt-sensitivity is a well-established phenomenon in hypertension, the underlying mechanisms of salt-sensitive hypertension are complex and still poorly understood. Studies have shown that there are multiple genetic factors associated

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with salt-sensitive hypertension, including the gene(s) associated with sodium-lithium countertransport (SLC) [1–5], a trait associated with the regulation of cell sodium that is highly heritable in primates [4]. Previous findings have suggested that dysregulation of the renin-angiotensin-aldosterone system (RAAS) may be involved in the mechanism linking elevated SLC activity and hypertension [6–8]. Findings by a number of groups indicate that an inappropriate maintenance of the RAAS function during sodium loading may lead to sodium retention, an alteration in the regulation of the movement of sodium across body fluid compartments, and an increase in cell sodium content [8–11]. Furthermore, blockade of the RAAS reverses the increase in SLC activity in hypertensive patients. Therefore, the goal of this study was to investigate the relationship between SLC activity, the RAAS, and the physiological regulation by sodium using a pedigreed, phenotyped and genotyped, salt-naïve baboon population.

Previous studies looking at the genetic aspects of hypertension and SLC in the baboon have shown that SLC activity is related to blood pressure (BP) on abnormal salt intake, that SLC activity is a heritable trait in the baboon, and that the gene for SLC activity is located on the baboon homologue of human chromosome 4 (baboon chromosome 5) [4]. For this study, baboons with decreased SLC activity (low SLC; LSLC) or increased SLC activity (high SLC; HSLC) were compared to a group of control animals with median or normal SLC activity (NSLC). The animals were given two diets differing in sodium content, with and without an angiotensin II (ANG II) infusion, to investigate the relationship among SLC activity, sodium regulation, and the RAAS. We anticipated that HSLC activity would be associated with inappropriate function of the RAAS and greater arterial pressure sensitivity to dietary sodium and ANG II and that LSLC activity would be associated with the least BP sensitivity. However, the LSLC phenotype was similar to the HSLC phenotype, and the NSLC phenotype showed the least BP sensitivity to dietary sodium and ANG II.

MATERIALS AND METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committee at Texas Biomedical Research Institute and conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care. The baboons used in this study were cared for in accordance with the U.S. Public Health Service Guide for the Care and Use of Laboratory Animals and the U.S. Animal Welfare Act.

Animal Selection

Red blood cell (RBC) SLC was measured in non-inbred, pedigreed male baboons (*Papio hamadryas*) from the Southwest National Primate Research Center colony as previously described [4] to select animals for the study. Fifteen adult male baboons were chosen: five with LSLC activity (SLC < mean − 1 SD for male baboons), five with NSLC activity (within ± 1 SD of mean SLC for male baboons), and five with HSLC activity (SLC > mean + 1 SD for male baboons). To ensure quality, samples were submitted as blind duplicates for analysis, and values for each group of analyses were considered acceptable when technical error was less than 15%.

Animal Procedures

Animals were fed a low-sodium diet (LS; 20 mmol sodium/500 g consumed and 90 mmol potassium/500 g) and tethered in individual metabolism cages for six to eight weeks before the study for acclimatization. Intravenous catheters were surgically implanted for 24-hour sampling of BP and intravenous infusion of ANG II, respectively. Following a two-week recovery, animals continued on the LS diet for six weeks (LS), with an ANG II infusion (1 ng/kg/min) incorporated during the latter half of the LS diet (LS+ANG). Animals were then switched to a high-sodium diet (HS; 150 mmol sodium/500 g consumed and 90 mmol potassium/500 g) with ANG II infusion (1 ng/kg/min) for three weeks (HS+ANG), followed by another three weeks on the HS diet without ANG II (HS). Thus, the study consisted of four consecutive phases, each three weeks in duration: LS, LS+ANG, HS+ANG, and HS.

Beat-to-beat measurement of systolic, diastolic, mean BP, and heart rate (HR) were averaged for every 10-minute period throughout the study using the Ponemah Physiology Platform signal conditioners and software (Data Sciences International, St. Paul, MN). BP and HR data reported here are based on the last seven days of each phase of the study (LS, LS +ANG, HS+ANG, and HS). Food intake, water intake, and urine output were measured during the first five and last five days of each study phase. Urine sodium and potassium concentrations were analyzed using a Corning flame photometer. Sodium intake was calculated as the sum of food intake plus the amount of sodium provided by the saline infusions to keep the catheters clear, and sodium output was calculated as the product of urine output and urine sodium concentration. Urinary aldosterone excretion was determined using an Aldosterone Coat-A-Count Kit (Diagnostic Products Corporation) as detailed below. Standard blood chemistry panels were performed throughout the study to measure hematocrit, glucose, total protein, blood urea nitrogen (BUN), and creatinine. Plasma concentrations of sodium and potassium were also determined with a Corning flame photometer.

Renal Function Studies

Renal function studies were performed two days after the initiation of each study phase to test the animals' ability to modulate renal blood flow, which was estimated using a steady-state infusion of p-aminohippurate (PAH); inulin was infused in a similar manner to estimate glomerular filtration rate (GFR) [12]. After collecting a control blood sample for plasma blank determination, loading doses of PAH (8 mg/kg) and inulin (50 mg/kg) were given, followed by a constant infusion containing PAH at a concentration that delivered 4 mg/min and inulin at a concentration that delivered 10 mg/min. Blood samples for steady-state plasma PAH and inulin concentration measurement were collected at 45, 60, and 90 minutes of each clearance period to ensure that steady-state plasma levels were achieved. Plasma PAH and inulin concentrations were measure by standard colorimetric procedures [13]. Renal clearances (ml/min) of PAH and inulin were then calculated by dividing the infusion rate (mg/min) by the steady-state plasma concentration (mg/ml).

Plasma Renin Activity and Aldosterone Concentration

Plasma renin activity (PRA) and plasma aldosterone concentration were measured by standard radioimmunoassay (RIA) procedures. PRA, which is defined as the rate of ANG I

generation from endogenous substrate, was measured using the Angiotensin I [^{125}I] Radioimmunoassay Kit (Dupont), and aldosterone was measured using the Aldosterone Coat-A-Count Kit (Diagnostic Products Corporation) according to the manufacturers' protocols. Both RIA procedures used three replicates for each dose of standard in the standard curve, and the standard curve was made linear with the logit b/b_0 transformation as recommended by Rodbard [14]. A least squares linear regression of logit b/b_0 versus log dose was fitted to the standard curve and used to calculate the amount of hormone in each sample assayed. All plasma samples were assayed in duplicate, and quality control of RIA procedures was achieved by including high- and low-quality control samples (Bi-level Plasma Control System, Ciba-Corning).

Statistical Analyses

Paired t-tests were used to measure the differences of each SLC phenotype across the different study phases, and unpaired t-tests were used to measure differences between the NSLC phenotype and the LSLC or HSLC phenotypes during the different study phases. Values were considered to be statistically significant if $p<0.05$. All data are expressed as mean±SEM.

RESULTS

Arterial Pressure and Heart Rate

Although there was a statistically significant difference in the mean SLC values between the three animal groups (LSLC, NSLC, and HSLC) used in the study ($p<0.0001$; Figure 1), there were no significant differences in arterial pressure between the NSLC animals and the LSLC or HSLC animals (Figure 2A). BP significantly increased in HSLC animals on the LS diet when ANG II was administered, while the LSLC and NSLC animals showed a significant BP increase when switched from the LS+ANG diet to the HS+ANG diet (Figure 2A). The largest difference in arterial pressures among the animal groups occurred after the ANG II infusion was discontinued on the HS diet; the BP of NSLC animals dropped back to the level it was on the LS diet while the BP of LSLC and HSLC animals remained elevated. The HR was quite different for the three SLC phenotypes (Figure 2B). The NSLC animals had a lower HR that fluctuated throughout the study while the HR of HSLC animals remained fairly steady at a higher rate, and the HR of LSLC animals was higher than NSLC and HSLC animals and increased further on the HS+ANG and HS diets.

Input/Output Summaries

HSLC animals consumed less food than the LSLC and NSLC animals, especially during the LS+ANG phase and start of the HS+ANG phase (data not shown). Conversely, water intake and urine output volumes were much higher for the HSLC animals with significantly higher amounts (compared to NSLC animals, $p<0.05$) at the start of the HS+ANG and HS phases (Figure 3A and 3C). An exception to this was a decrease in water intake at the beginning of the LS+ANG diet. The sodium intake and output were similar for the three groups of animals, except the LSLC animals had increased sodium output compared to the NSLC and HSLC animals at the beginning of the HS diet (Figure 3B and 3D). However, HSLC animals showed the highest level of plasma sodium concentration throughout the study, followed by

LSLC and then NSLC animals had the lowest concentration. The amount of aldosterone excreted in the urine was similar for the three SLC phenotypes until the end of the HS+ANG phase, where the HSLC animals had a lower amount of aldosterone excretion compared to NSLC and LSLC animals. However, the NSLC animals had the lowest level of aldosterone excretion on the HS diet, and the LSLC animals had a much higher level of aldosterone excretion (HSLC animals had a slightly higher amount of excretion than the NSLC animals) (data not shown).

Renal Function

PAH clearance was marginally higher in NSLC animals compared to LSLC and HSLC animals during the LS phase of the study and increased slightly in all three SLC phenotypes when ANG II was administered on the LS diet. It then increased in LSLC animals during the HS+ANG phase but dropped slightly in NSLC and HSLC animals. The rate of PAH clearance was similar for the three SLC phenotypes on the HS diet without ANG II, but it was slightly higher in the NSLC animals (data not shown). The rate of inulin clearance was lowest in the HSLC animals throughout the study. All three phenotypes showed an increase when ANG II was administered on the LS diet followed by a drop during the HS+ANG phase, with the rate being higher in the LSLC animals. The rate of inulin clearance then increased slightly on the HS diet without ANG II infusion in the LSLC and NSLC animals but decreased in the HSLC animals (data not shown).

PRA and Aldosterone

PRA was higher in NSLC animals than LSLC and HSLC animals on the LS diet. It significantly decreased in NSLC and LSLC animals when the ANG II infusion was added on the LS diet and then increased on the HS+ANG and HS diets, with an increase in LSLC animals (Figure 4A). PRA also decreased in HSLC animals when the ANG II infusion was added on the LS diet, but it continued to decrease on the HS+ANG diet and, like LSLC animals, increased on the HS diet (Figure 4A). NSLC animals had the highest concentration of aldosterone in the plasma, which then decreased when the ANG II was administered on the LS diet and then again on the HS diet. The aldosterone concentration in HSLC animals showed an opposite trend. It increased during the LS+ANG phase, decreased during the HS +ANG phase, and then increased again during the HS phase. LSLC animals had an intermediate level of aldosterone in the plasma on the LS diet, which then continued to decrease throughout the study (Figure 4B).

CONCLUSIONS

The factor(s) responsible for the onset of salt-sensitive hypertension are not yet known. One reason may be that there are several factors contributing to the development of the hypertension [15]. Two initiating factors that have emerged strongly over the years are genetics and altered renal function. Unfortunately, neither factor has been associated with a single mechanism that could be identified as the single cause for all salt-sensitive hypertension. This is probably a testament to the complexity of the pathophysiology. The early work of Guyton and colleagues has suggested that a reduced renal function has a pivotal role in the manifestation of hypertension [16]. Since then, Hall et al. have shown the

importance of the rise in renal perfusion pressure accompanying the increase in arterial pressure in re-establishing sodium balance and limiting the extent of the hypertension [17]. However, other than physical factors interfering with renal function (occlusion of the renal arteries, renal compression, reduced renal mass) or disease states (such as glomerulosclerosis), it is not clear how a reduced renal function occurs. Evidence has emerged supporting the view that genetic factors can contribute to the development of salt-sensitive hypertension.

Previous studies have shown that HSLC activity, a trait that is highly heritable in primates [4], is associated with the occurrence of hypertension [1,18–24]. This phenotype is also associated with an increased or unchanged intracellular sodium, as well as sodium sensitivity to BP [8,22,25]. Therefore, we wanted to address whether baboons with distinctly different genetically-determined phenotypes for SLC activity were predisposed to mechanisms that are thought to be involved in salt-sensitive hypertension, such as an inappropriately active RAAS during an increase in body sodium. Animals were given a HS diet to determine the response of LSLC, NSLC, and HSLC activity animals with respect to renal function and BP sensitivity to sodium to establish whether the baboons with the genetic HSLC phenotype showed the same dysregulation of the RAAS as humans. We also investigated the contribution of peripheral angiotensin during a LS and HS diet to provide mechanistic insights into the relationship between the SLC phenotype, sodium, and the RAAS.

We speculated that animals with HSLC activity would be most sensitive to the hypertensive effects of sodium and angiotensin due to an impaired ability to maintain cellular sodium balance and that animals with LSLC activity would be the least sensitive. However, we were surprised to find that the NSLC phenotype appeared to be the least sensitive to sodium and angiotensin throughout the study, and the LSLC phenotype appeared to be just as sensitive as the HSLC phenotype. In fact, the BP of the LSLC group was slightly higher than that of the HSLC animals on the HS+ANG and HS diets. Furthermore, the rate and concentration of PRA and aldosterone, respectively, were elevated in both the LSLC and HSLC animals on the HS diet. This could indicate an inappropriately elevated level of RAAS, consistent with human studies of HSLC patients. However, ANG II can have lasting effects, even after cessation, and the PRA levels are highly variable in the LSLC and HLSC animals on the HS diet. Therefore, these results should not be over-interpreted and require further investigation.

Although we saw higher arterial pressures in HSLC baboons during the LS+ANG and HS phases of the study, we expected a greater response to the ANG II and HS challenges. While SLC occurs primarily in RBCs, there are other mechanisms (such as Na-K ATPase, Na-Ca exchange, and Na-H exchange) found in other kinds of cells that are also activated by increases in sodium concentration. So it is possible that the defect in the SLC may also be associated with other sodium transport mechanisms, which were not addressed in this study. Also, the SLC phenotype of the animals was based on activity measurements in RBCs. It should be noted that SLC-like activity has been documented in other cells types, such as lymphocytes and skin fibroblasts. This could help explain the lack of a “dose response” with regard to SLC expression. Furthermore, animals with HSLC activity had a much greater

level of water intake and urine output, which may have mitigated the effects of the ANG II infusion and HS challenge.

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Highlights

- Sodium-lithium countertransport (SLC) activity is highly heritable in primates.
- Increased SLC activity has been associated with salt-sensitive hypertension.
- Baboons were fed low- and high-salt diets with and without angiotensin II (ANG II).
- Low SLC correlated with blood pressure sensitivity similar to high SLC phenotype.
- Normal SLC phenotype showed least blood pressure sensitivity to salt and ANG II.

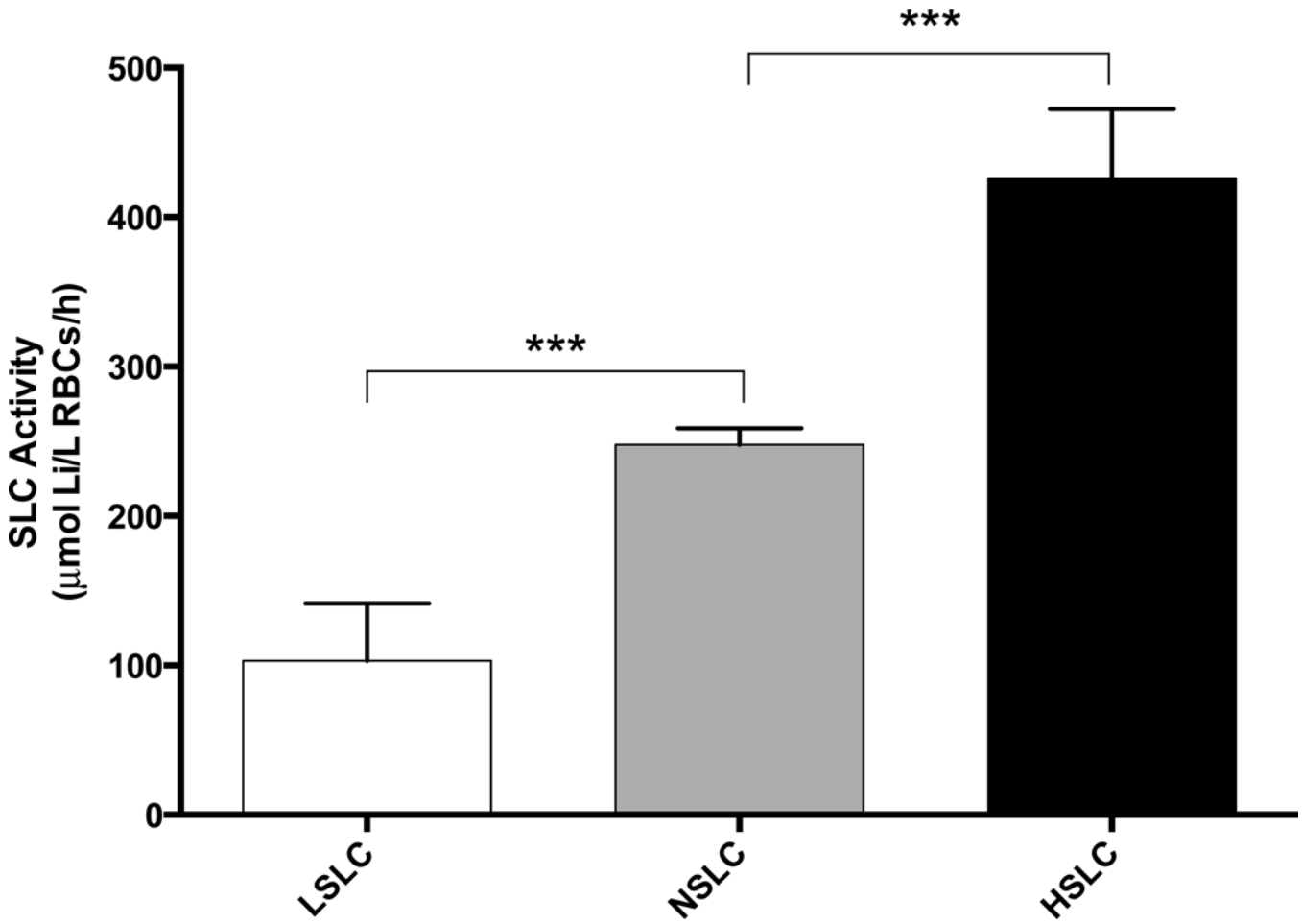

Figure 1. SLC activity

Mean SLC values among the three animal groups were significantly different (***p<0.0001). Data are shown as mean ± SEM. SLC= sodium-lithium countertransport; LSLC= low sodium-lithium countertransport; NSLC= normal sodium-lithium countertransport; HSLC= high sodium-lithium countertransport; µmol Li/L RBCs/h= micromoles of lithium per liter of red blood cells per hour.

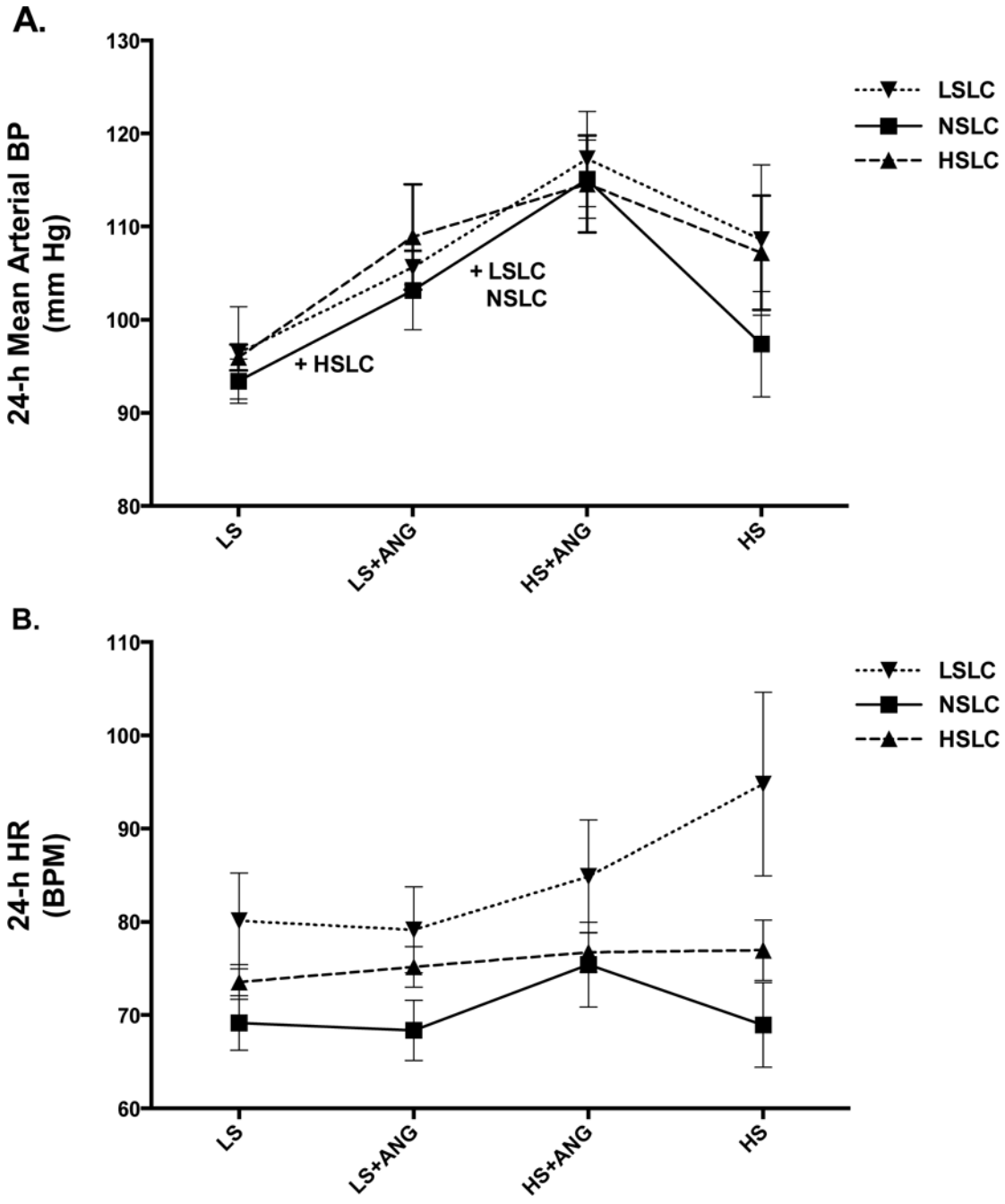

Figure 2. 24-h mean BP and HR averaged for the last seven days of each study phase

A) Arterial BP and B) HR for each group; "+" indicates a significant difference for the SLC group across the different study phases. BP= blood pressure; mm Hg= millimeters of mercury; HR= heart rate; BPM= beats per minute; LSLC= low sodium-lithium countertransport; NSLC= normal sodium-lithium countertransport; HSLC= high sodium-lithium countertransport; LS= low salt; LS+ANG= low salt with angiotensin II; HS+ANG= high salt with angiotensin II; HS= high salt.

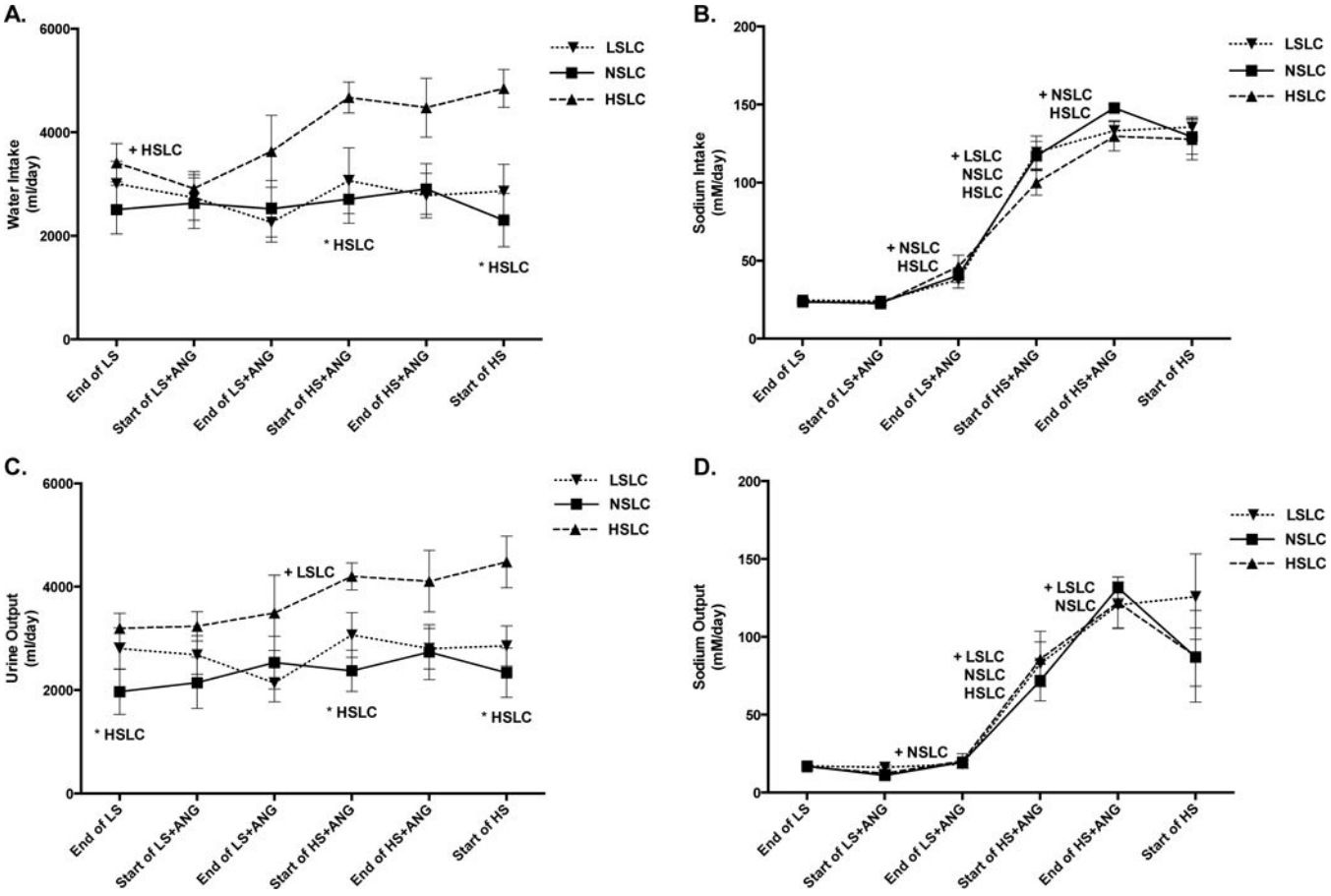

Figure 3. Input/Output Summaries

A) Water intake; B) Sodium intake; C) Urine output; and D) Sodium output. "+" indicates a significant difference for the SLC group across the different study phases, $p<0.05$; * indicates significant differences between the NSLC phenotype and the LSLC or HSLC groups during the different study phases, $p<0.05$. ml/day= milliliters per day; mM/day= millimoles per day; LSLC= low sodium-lithium countertransport; NSLC= normal sodium-lithium countertransport; HSLC= high sodium-lithium countertransport; LS= low salt; LS +ANG= low salt with angiotensin II; HS+ANG= high salt with angiotensin II; HS= high salt.

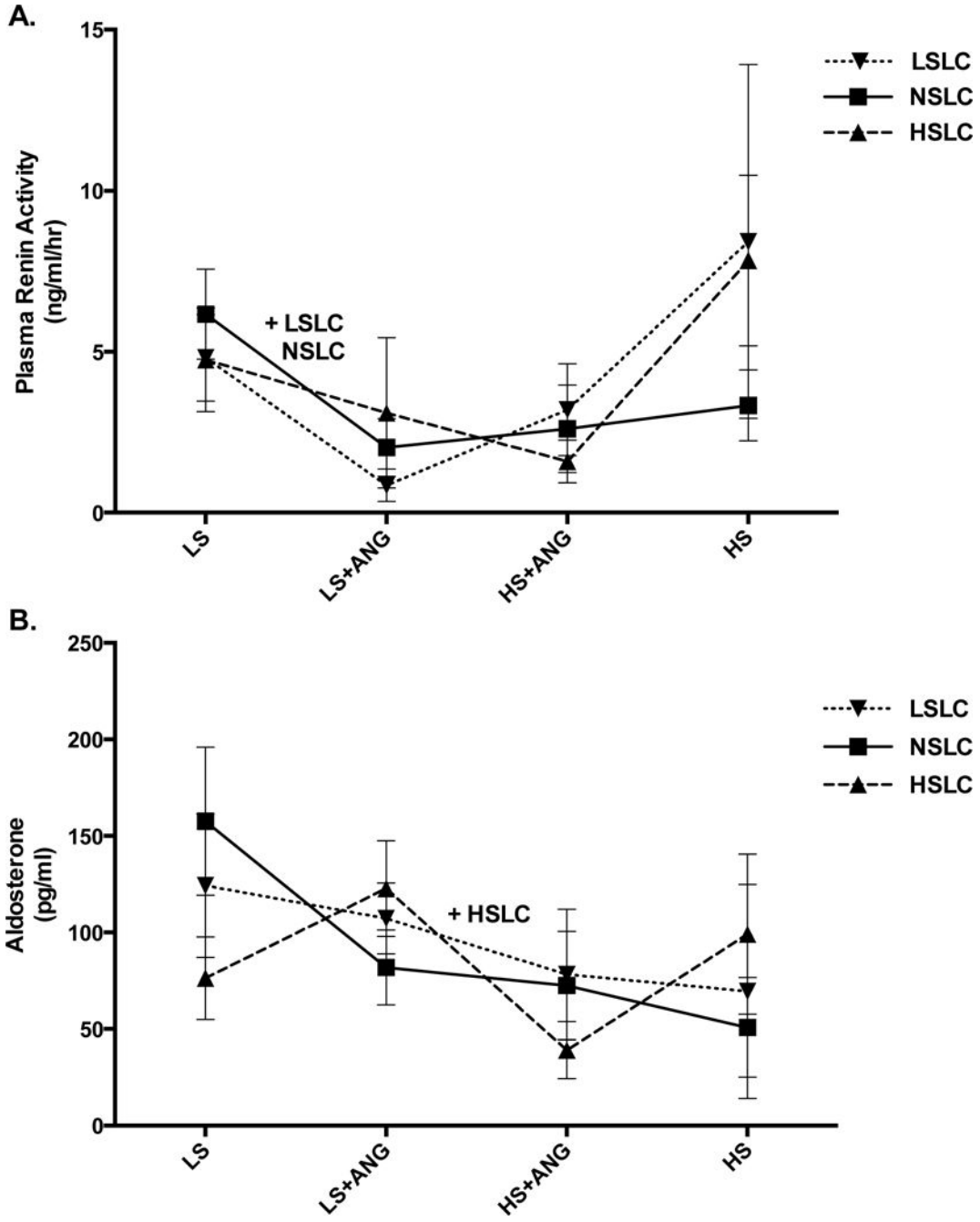

Figure 4. Plasma Renin Activity and Aldosterone Measurements

A) PRA and B) Aldosterone for each group. "+" indicates a significant difference for the SLC group across the different study phases. ng/ml/hr= nanograms per milliliter per hour; pg/ml= pictograms per milliliter; LSLC= low sodium-lithium countertransport; NSLC= normal sodium-lithium countertransport; HSLC= high sodium-lithium countertransport; LS= low salt; LS+ANG= low salt with angiotensin II; HS+ANG= high salt with angiotensin II; HS= high salt.