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SELECTIVE CHLORIDE LOADING IS PRESSOR IN THE STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT DESPITE HYDROCHLOROTHIAZIDE-INDUCED NATRIURESIS

Olga Schmidlin[†], Masae Tanaka[†], Anthony Sebastian, and R. Curtis Morris Jr

University of California San Francisco, Department of Medicine, Division of Nephrology, San Francisco, CA

Abstract

Objective—To test the hypothesis that in the stroke-prone spontaneously hypertensive rat (SHRSP), the pressor effect of selective dietary chloride loading depends on a positive external sodium balance.

Methods—In 43 male SHRSP fed a Japanese style diet containing a low normal amount of NaCl (0.4%) we compared the effects on telemetrically measured systolic blood pressure (SBP) of hydrochlorothiazide, 25 mg/kg per day, alone (“TZ”, n=11); hydrochlorothiazide combined with either KCl (“KCLTZ”, 2%K, n=10) or KHCO₃ (“KBCTZ”, 2%K, n=11) and no hydrochlorothiazide (“CTL”, n=11) over a 10-week period starting at 10 weeks of age.

Results—With either TZ or KBCTZ, SBP did not increase above baseline values. However, KCLTZ induced a sustained increase in SBP of 17 mmHg (p<0.0001), an increase almost half that occurring without hydrochlorothiazide (CTL), 38 mmHg (p<0.0001). Such divergence of blood pressures with KCLTZ and KBCTZ began over the first three days of their administration, even while they induced similarly negative external sodium balances, a positive one occurring only in CTL. Body weight increased more without, than with, hydrochlorothiazide, but did not differ between KCLTZ and KBCTZ. Changes in SBP occurring on the 2nd day after treatment assignment predicted final changes.

Conclusions—These results demonstrate that in the SHRSP, dietary KCl loading can induce a pressor effect despite concomitant hydrochlorothiazide-induced natriuresis that elicits a negative external sodium balance. The results provide evidence that in the SHRSP the pressor effect of selective chloride loading does not depend on a positive external sodium balance, but rather on a mechanism actuated by chloride per se.

Keywords

Chlorides; natriuresis; hypertension; physiopathology; thiazide diuretics; rats

Correspondence: Olga Schmidlin, MD, University of California San Francisco, 1291 Moffitt Hospital, Box 0126, San Francisco, CA 94143-0126, USA; Telephone: (415) 476-502-4364, (415) 476-4336, Fax: (415) 476-6264; olga@picasso.ucsf.edu; yfay@medicine.ucsf.edu.

[†]These authors contributed equally to this report

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Introduction

Blood pressure can vary directly with the dietary intake of NaCl. A pathophysiological mechanism of such “salt sensitivity” has not been defined, but the prevailing hypothesis holds that a single final common pathway mediates all pressor effects of dietary NaCl: An abnormally enhanced renal reclamation of NaCl causes positive external balances of both sodium and chloride, which jointly mediate an osmotic expansion of plasma volume that evokes an increase in cardiac output, the proximate cause of all pressor effects of dietary NaCl.¹⁻⁴ In accord with this hypothesis, in most instances of salt sensitivity, selective dietary loading of either sodium or chloride has failed either to induce or amplify a pressor effect.⁵⁻⁸ Yet, three exceptions are reported: In particularly salt-sensitive normotensive African Americans (blacks), selective sodium loading with NaHCO₃ induced a pressor effect despite sustained chloride restriction.⁹ In the spontaneously hypertensive rat (SHR), selective chloride loading with choline and glycine chloride combined induced a pressor effect much like that induced by dietary NaCl, but also systemic toxicity.¹⁰ In the stroke-prone SHR (SHRSP), a more salt-sensitive genetic derivative of the SHR, dietary loading of KCl, both alone and combined with NaCl, induced pressor effects whose extent varied directly, dose-dependently and only with the chloride load.^{11, 12} However, given that dietary NaCl was at least normal in these chloride loading studies, an undetected positive external sodium balance may have been required for their apparently chloride-mediated pressor effects. If not, in the SHRSP, KCl might induce a robust pressor effect even as hydrochlorothiazide induced a reduction in the renal reclamation of sodium, a negative external balance of sodium and an attenuation of hypertension, inductions we now report.

Methods

Male SHRSP (University of Iowa, Iowa City, Iowa) were fed a Japanese style diet (0.5% K⁺ and 0.4% NaCl; Zeigler Bros., Inc., Gardners, Pennsylvania) from 6 weeks of age. Until randomization, but not afterwards, this diet was supplemented in all rats with 1.5% K⁺ supplied as KHCO₃. At 10 weeks, rats were randomly assigned to 4 groups: (a) hydrochlorothiazide alone (“TZ”, 25 mg of hydrochlorothiazide per kg BW per day, n=11); (b) hydrochlorothiazide combined with KCl (“KCLTZ”, 2%K, n=10); or (c) with KHCO₃ (“KBCTZ”, 2%K, n=11); and (d) no hydrochlorothiazide (“CTL”, n=11). Diet electrolyte composition and food and electrolyte intakes are summarized in Table 1. The amount of chloride provided as KCl in the current study has been shown to increase significantly plasma levels of chloride in the SHRSP.^{11, 13} Hydrochlorothiazide, 25 mg per kg BW per day, was shown to attenuate the increase in blood pressure and to extend life span in the SHRSP.¹⁴ Increasing the dose to 50 mg per kg BW per day did not amplify either effect.¹⁴ Deionized drinking water was supplied ad libitum. All groups were fed and housed as before,¹¹ and studied according to the guidelines of the University of California, San Francisco, Committee on Animal Research.

In each rat at 8 weeks of age we implanted intraperitoneally a radiotelemetric blood pressure-measuring device with a pressure-sensing catheter inserted into the infrarenal aorta (model TA11PA; Data Sciences International, St. Paul, Minnesota).¹¹ In each rat, we calculated successive mean daily and weekly values of systolic and diastolic blood pressure (SBP and DBP, respectively) from measurements obtained over 5-second intervals every 10 minutes. The main outcomes were the average changes in SBP during days 1 to 3 days (“initial” change) and weeks 8 to 10 (“final” change) after assignment.

Body weight (BW) of each rat was measured weekly. 24-hr urinary excretion rates of sodium (UNaV), chloride (UCIV), creatinine (UcrV), and total protein (UPV) were measured at baseline (age 9 weeks) and on days 1, 2, 3, and during week 4, 8 and 10 after

assignment. Sodium balance was calculated as the difference of urinary output and dietary intake. For longitudinal analysis UcrV values were adjusted for BW.

For statistical analysis we employed the “Statistica” software package (Statsoft Inc., Tulsa, Oklahoma). We used paired t-tests to assess within-group differences of variables. To assess between-group differences in Δ SBP, Δ UNaV, sodium balance, Δ UcrV and other variables we used ANOVA followed by the Tukey and Newman-Keuls test. To assess interrelationships between variables we used linear regression analysis. Data are presented as mean and 95% confidence intervals (C.I.). A *P* value of <0.05 was considered statistically significant.

Results

Baseline blood pressure

In 9 to 10 week old rats, before assignment, blood pressure was similar in all groups (Table 2). Blood pressure values showed circadian variation with average baseline day time SBP being about 7 mmHg lower than average night time SBP. Throughout the study, changes in SBP and DBP in individual rats varied with each other directly and highly significantly ($R=0.816$ and 0.776 , respectively, in week 1 and throughout weeks 8 to 10; $P<0.0001$ for either comparison). Therefore, SBP values only were used for analyses.

Antipressor effect of hydrochlorothiazide given alone or in combination with either KCl or KHCO_3 : Comparison to an untreated control group

In untreated rats (CTL), SBP did not change significantly during the first 3 days after assignment (Figure 1). Thereafter, it increased progressively at a rate of almost 4 mmHg per week. “Final” SBP in CTL was 38 (30/46) mmHg (22 [18/26] %) above baseline (Figure 2).

As in CTL, SBP in rats given hydrochlorothiazide plus KCl (KCLTZ) did not change from baseline values during the first 3 days after assignment (Figure 1). Thereafter, SBP increased by about 2 mmHg per week. “Final” SBP in KCLTZ was 17 (13/21) mmHg (10 [8/12] %) above baseline (Figure 2). The “final” increase in SBP in KCLTZ was significantly greater than that in TZ and KBCTZ (see below) and significantly smaller than that in CTL.

In rats given hydrochlorothiazide alone (TZ) or in combination with KHCO_3 (KBCTZ), the extent and time course of BP changes were similar (Figures 1 and 2). Both treatments abolished progression of hypertension. In fact, throughout the first 3 days after assignment, SBP of TZ and KBCTZ groups, *decreased* significantly from baseline values, as previously reported,¹⁴ by a maximum of -11 (-13/-9) and -9 (-11/-7) mm Hg, respectively. The decreases were sustained throughout the 1st week. After the 1st week, SBP values in TZ and KBCTZ groups returned to values not different from those at baseline and remained at these levels throughout the remainder of the study. Both “initial” and “final” SBP changes in TZ and KBCTZ differed significantly from those in CTL and KCLTZ. For all groups combined, changes in SBP on day 2 after assignment, but not baseline SBP, were highly predictive of “final” changes (Figure 3).

Sodium excretion and external sodium balance

At baseline, UNaV (mmol/d) and external sodium balances did not differ between groups (Table 3). Sodium balances were positive in all groups (Table 3). Over the first 3 days after assignment, average UNaV increased significantly in all groups receiving hydrochlorothiazide, by 20 to 30%, but not in CTL (Table 3 and Figure 4). Cumulative sodium balance over the first 3 days after assignment became similarly negative in KCLTZ and in KBCTZ, -0.4 (-0.8/0) and -0.3 (-0.7/0.1) mmol, respectively. In TZ, cumulative

sodium balance became neutral, 0.3 (-0.1/0.7) mmol and in CTL it remained positive, 0.7 (0.3/1.1) mmol. During weeks 4 to 10 after assignment, average UNaV was significantly greater than baseline UNaV in all groups (Table 3) and the increase in UNaV from baseline was greater in KCLTZ than in the other 3 groups, ANOVA $P < 0.01$.

Chloride excretion

At baseline, UCIV did not differ between groups (Table 3). Over the first 3 days after assignment, average UCIV increased significantly in all groups receiving hydrochlorothiazide, but not in CTL. Consistent with the increased chloride intake, the increase of UCIV in KCLTZ was much greater, 530%, than in either KBCTZ, 32%, or in TZ, 24%.

Body weight

Baseline BW was similar in all groups (Table 2). In all groups, BW increased progressively and significantly over the 10-week study period (Figure 5). In the first week after assignment, BW increased by: 8% (6/10) in CTL, 6% (4/8) in KCLTZ and 4% (3/5) in both TZ and KBCTZ. The increase in CTL was significantly greater than that in TZ and KBCTZ. Increases in KCLTZ and KBCTZ were not different. Throughout the remainder of the study, increases in CTL were significantly greater than those in the other 3 groups, and increases in KCLTZ and KBCTZ were not different.

Urine creatinine excretion

UcrV at baseline was similar in all groups (Table 2). Immediately after assignment, UcrV, adjusted for 100 g BW, decreased significantly and remained at significantly reduced levels throughout the study in all groups except in KBCTZ (Figure 6).

Urine protein excretion

UPV at baseline was similar in all groups (Table 2) and did not increase in any of the groups during the first 4 weeks of the study (Figure 7). Thereafter, UPV increased significantly above baseline values in KCLTZ and CTL but not in TZ and KBCTZ.

Heart rate

Heart rates were recorded at baseline and during the first 3 days after assignment. Average 24-hour heart rates at baseline were similar in all groups. After assignment heart rates decreased in TZ on days 1 through 3 by almost 3% and in KCLTZ and KBCTZ on day 2 by about 1.5% (Table 4).

Discussion

The current observations confirm that in the SHRSP, dietary KCl, but not KHCO_3 , induces a robust sustained pressor effect.¹¹ Further, these observations demonstrate that in this rat, selective chloride loading with KCl induces a sustained pressor effect even as hydrochlorothiazide is concomitantly administered at a dose that induces an increased urinary excretion of sodium and a sustained attenuation of hypertension. Indeed, whether administered alone or together with KHCO_3 , hydrochlorothiazide immediately abolished the progression of hypertension, as previously described in the SHRSP.¹⁴ This is the first demonstration that the dietary loading of any salt other than NaCl can thwart or reverse the anti-pressor effect of hydrochlorothiazide. The pressor effect of NaCl has generally been attributed to its induction of parallel increases in the mutually complementary extracellular osmotic activities of sodium and chloride, and to their joint mediation of an expansion of plasma volume,¹⁻⁴ an expansion that can be contracted by the natriuretic and chloruretic

renal tubular effects of hydrochlorothiazide.¹⁵ But it would seem unlikely that the pressor effect of KCl loading could be mediated by an osmotic expansion of plasma volume. In body water, the great bulk of potassium, unlike that of sodium and chloride, is not distributed in extracellular fluid, and is therefore not a major determinant of plasma osmotic activity. Further, potassium has a natriuretic effect, and also an anti-pressor effect, as observed in the SHRSP when KHCO_3 alone was loaded.¹¹ The capacity of KCl to thwart the antipressor effect of hydrochlorothiazide must then involve some pressor agency of chloride.

From the outset of its loading, KCl greatly attenuated the antipressor effect of hydrochlorothiazide. Indeed, over the first three days of loading, KCl abolished the striking decreases in SBP otherwise immediately induced by hydrochlorothiazide, the values of SBP with KCl remaining similar to those observed in CTL rats, i.e. those not given hydrochlorothiazide. By contrast, KHCO_3 did not alter the antipressor effect of hydrochlorothiazide, the decreasing values of SBP with KHCO_3 being indistinguishable from those with hydrochlorothiazide alone. The divergent effects of KCl and KHCO_3 on the antipressor effect of hydrochlorothiazide cannot be related to divergent metabolic effects, at least not to those traditionally viewed as causally related to the pressor effect of NaCl. Thus, that only KCl abolished the antipressor effect of hydrochlorothiazide cannot be related to a sodium balance more positive, or a plasma volume more expanded, than any occurring with KHCO_3 loading. Specifically, over the first three days of loading, sodium balance was positive only in control rats, and similarly negative in rats given hydrochlorothiazide with either KCl or KHCO_3 , in keeping with the natriuretic effect of hydrochlorothiazide. In keeping with a continuance of this metabolic effect throughout the course of the study, similar gains in weight occurred in rats given hydrochlorothiazide, whether combined with either KCl or KHCO_3 .

These metabolic observations comport with ones made in chloride-loading studies of the SHRSP not given hydrochlorothiazide: Whether the pressor effect of NaCl loading was exacerbated by concomitant KCl loading or unaffected by concomitant KHCO_3 loading, similar increases in external sodium balance and body weight occurred in all three NaCl-loaded groups.¹² In the absence of NaCl loading, when KCl loading induced a sustained pressor effect, and KHCO_3 loading induced a sustained antipressor effect, relative to the levels of blood pressure occurring without loading of either potassium salt, apparent sodium balances were similar in all three groups, and body weights increased similarly.¹¹ Thus, in the SHRSP studied under a variety of metabolic conditions that have given rise to a broad range of external sodium balances, dietary chloride loading has induced pressor effects that cannot be related to positive sodium balances, but only to the chloride load imposed. In accord with these earlier studies, the early divergence of SBP in the current study cannot be ascribed to divergent renal structural integrities, as urinary excretion of protein had not increased four weeks after experimental assignment, when half of the pressor effect to be attained in KCLTZ rats had already occurred. Only later, when urinary excretion of protein did increase, does it seem likely that selective chloride loading could induce a renal structural abnormality, e.g. microangiopathy,¹³ that might contribute to the pressor effect of chloride.

In the current study, the changes in SBP occurring on the 2nd day after treatment assignment strongly predicted their final changes, when KCl loading induced a pressor effect that effectively halved the antipressor effect of hydrochlorothiazide, with and without KHCO_3 loading. In previous studies of the SHRSP not given hydrochlorothiazide, but loaded with either KCl or KHCO_3 , with and without concomitant NaCl loading, similarly divergent changes in SBP occurred early and strongly predicted later ones.^{11, 12} That such early changes in SBP could be predictive of such divergence, and in the absence of divergence in external sodium balance, suggests that a common mechanism mediates changes in blood

pressure from their outset, and through a mechanism that does not depend on changes in the external sodium balance.

This suggestion is clearly at odds with the prevailing formulation of the pressor effect of dietary NaCl,¹⁻⁴ but not at odds with our previously formulated pressor mechanism of dietary NaCl in the SHRSP.^{11, 12} In accord with our previous observations in studies of the SHRSP conducted without administration of hydrochlorothiazide,^{12, 13} in the current studies of SHRSP dietary loading of KCl, but not KHCO₃, induces a near immediate increase in urinary excretion of chloride and a near immediate decrease in urinary excretion of creatinine, which likely reflects a decrease in glomerular filtration rate. KCl-induced chloruresis presumably reflects an increased delivery of chloride into the distal renal tubule and to the macula densa of its thick ascending limb, where an increased delivery of chloride and its reclamation elicits constriction of the afferent renal arteriole as part of the normal tubular glomerular feedback response.¹⁶ It seems likely that the abnormal enhancement of this response demonstrated in the SHR is pathogenic of hypertension,^{17, 18} and a mediator of the pressor effect currently observed with selective chloride loading. It is to be noted that the increased urinary excretion of sodium and chloride induced by hydrochlorothiazide reflects their decreased reclamation in the distal convoluted renal tubule, not in the preceding thick ascending limb.¹⁵

Given that in the SHRSP selective chloride loading can elicit a pressor effect without an apparent positive sodium balance,^{11, 12} and despite a concomitant negative external sodium balance as shown in the current study, the chloride component of dietary NaCl could in some instances of salt sensitivity account mainly if not entirely for the pressor effect of NaCl, irrespective of its component ions' joint capacity to determine plasma volume expansion. In fact, the general assumption that the plasma volume expansion induced by dietary NaCl determines its pressor effect has recently been called into question by several studies of salt sensitivity in both humans and animals.^{9, 19-21} In a study comparing normotensive salt-sensitive and salt-resistant blacks with respect to the time courses of their hemodynamic and metabolic responses to NaCl loading, there occurred similar increases in external sodium balance, plasma volume and cardiac output.¹⁹ However, the divergent changes in mean arterial blood pressure (MAP) were attended by divergent changes in systemic vascular resistance (SVR). Whereas SVR changed little over the first three days of NaCl loading in the salt-sensitive, over this period in the salt-resistant, SVR decreased sharply, along with MAP, both variables thereafter increasing progressively toward baseline. In the salt-sensitive blacks, it is the impaired decrease in SVR that rendered pressor the normal NaCl-induced increase in cardiac output. In all subjects combined, the changes in SVR and MAP induced by NaCl on the 2nd day of its loading were strongly predictive of the changes in MAP induced on the 7th day of NaCl loading. NaCl induced changes in plasma volume and cardiac output were not predictive. In another recent study of mostly normotensive blacks, NaHCO₃ loading induced a substantial pressor effect in the most salt-sensitive, despite inducing an increase in plasma volume only a fraction of that induced by NaCl in salt-resistant subjects.⁹

It seems likely that dietary NaCl can induce a pressor effect by more than one pathophysiological mechanism. It has long been held that in order for dietary NaCl to induce a pressor effect, the sequential occurrence of a positive external NaCl balance, a plasma volume expansion and an increase in cardiac output is a necessary and sufficient pathogenic sequence. However, in many salt-sensitive normotensive blacks, it seems likely that this sequence is a necessary but not a sufficient pathogenic sequence to induce a pressor effect. In the currently studied SHRSP, as in an inbred Dahl S strain,²⁰ it would seem that the sequence is *not* necessary for dietary NaCl to induce a pressor effect.

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Figure 1. “Initial” changes in SBP in SHRSP treated with either hydrochlorothiazide alone (TZ) ◆; hydrochlorothiazide plus dietary KCl (KCLTZ) ●; hydrochlorothiazide plus dietary KHCO_3 (KBCTZ) ▲; or no treatment (CTL) ■. Within-group comparison: In CTL and KCLTZ, SBP did not change significantly from baseline values during the initial 3 days after assignment. By contrast, in TZ and KBCTZ, SBP *decreased* significantly. Between-group comparison of Δ SBP on day 3 after assignment: ANOVA $P < 0.0001$; Δ SBP in CTL differs significantly from Δ SBP in the other 3 groups. Δ SBP in KCLTZ differs significantly from Δ SBP in TZ and in KBCTZ. * denotes significant difference from CTL; † denotes significant difference from KCLTZ, $P < 0.05$; values are mean and 95% C.I. KCLTZ, $n=10$; other groups, $n=11$.

Figure 2.

Time course of changes in SBP during 10 weeks of treatment with either hydrochlorothiazide alone (TZ) ◆; hydrochlorothiazide plus dietary KCl (KCLTZ) ●; hydrochlorothiazide plus dietary KHCO₃ (KBCTZ) ▲; or no treatment (CTL) ■. In CTL, SBP increases progressively by almost 4 mmHg per week to a “final” increase (average of weeks 8 to 10) 38 mmHg above baseline. In KCLTZ, SBP increases by about 2 mmHg per week to a “final” increase of 17 mmHg above baseline. In TZ and KBCTZ SBP does not increase significantly above baseline values. Between-group comparison of “final” ΔSBP (average of weeks 8,9 and 10): ANOVA $P < 0.00001$; Newman Keuls test: * denotes

significant difference from CTL; † denotes significant difference from KCLTZ; values are mean and 95% C.I. KCLTZ, n=10; other groups, n=11.

Figure 3. Relationship between changes in SBP on day 2 after assignment and "final" changes. For all groups combined, the "initial" change in SBP is highly predictive of the "final" change.

Figure 4.

Changes in urinary sodium excretion (UNaV) in SHRSP treated with either hydrochlorothiazide alone (TZ); hydrochlorothiazide plus dietary KCl (KCLTZ); hydrochlorothiazide plus dietary KHCO_3 (KBCTZ); or no treatment (CTL). During the first 3 days after assignment, UNaV increased significantly in all hydrochlorothiazide-treated groups, by 20 to 30%, but not in CTL. Shown is average change of UNaV of days 1, 2 and 3; * denotes significant difference from baseline, $P < 0.05$; mean and 95% C.I. KCLTZ, $n=10$; other groups, $n=11$.

Figure 5.

Time course of changes in body weight (BW) during 10 weeks of treatment with either hydrochlorothiazide alone (TZ) ◆; hydrochlorothiazide plus dietary KCl (KCLTZ) ●; hydrochlorothiazide plus dietary KHCO_3 (KBCTZ) ▲; or no treatment (CTL) ■. In all groups, BW increased progressively and significantly over the 10-week study period. Between-group comparison: Week 1, ANOVA $P < 0.001$. * The increase in CTL was significantly greater than that in TZ, KCLTZ and KBCTZ. Increases in KCLTZ and KBCTZ were similar. Average of weeks 8 to 10, ANOVA, $P = \text{ns}$. Values are mean and 95% C.I. KCLTZ, $n = 10$; other groups, $n = 11$.

Figure 6.

Time course of changes in urinary creatinine excretion (UcrV) during 10 weeks of treatment with either hydrochlorothiazide alone (TZ) ◆; hydrochlorothiazide plus dietary KCl (KCLTZ) ●; hydrochlorothiazide plus dietary KHCO_3 (KBCTZ) ▲; or no treatment (CTL) ■. Values are adjusted for BW. In all groups except in KBCTZ UcrV decreased significantly from baseline values. The decrease occurred within the first 3 days after assignment and persisted throughout the study. Within-group comparison: * denotes significant difference from baseline. Between-group comparison Day 3, ANOVA $P < 0.000001$; average of weeks 8 and 10, ANOVA $P < 0.00001$. Newman Keuls test: † denotes significant difference from CTL, TZ and KCLTZ. Values are mean and 95% C.I. KCLTZ, $n=10$; other groups, $n=11$.

Figure 7.

Time course of changes in urinary protein excretion during 10 weeks of treatment with either hydrochlorothiazide alone (TZ) ◆; hydrochlorothiazide plus dietary KCl (KCLTZ) ●; hydrochlorothiazide plus dietary KHCO₃ (KBCTZ) ▲; or no treatment (CTL) ■. Within-group comparison of average values of weeks 8 and 10: * denotes significant difference from baseline. Between-group comparison of average values weeks 8 and 10: †, KCLTZ and CTL differ significantly from TZ and KBCTZ. Values are mean and 95% C.I. KCLTZ, n=10; other groups, n=11.

Table 1**Food and Electrolyte Composition and Intake**

Group	TZ	KCLTZ	KBCTZ	CTL
Treatment	HCTZ*	HCTZ*+KCl	HCTZ*+KHCO ₃	No Rx
n	11	10	11	11
<i>Dietary electrolyte composition, mmol/100g chow (% weight)</i>				
Diet Na⁺	7 (0.4)	7 (0.4)	7 (0.4)	7 (0.4)
Diet Cl⁻	7	44	7	7
Diet K⁺	13 (0.5)	51 (2.0)	51 (2.0)	13 (0.5)
<i>Daily food intake, g per day, average of weeks 0 to 10 (95% C.I.)</i>				
Food Intake[†]	20.1 (19.6/20.7)	20.7 (19.9/21.6)	20.7 (19.8/21.5)	20.1 (19.3/20.9)
<i>Daily dietary electrolyte intake before assignment (Week 0), mmol/day (95% C.I.)</i>				
Diet Na⁺	1.5 (1.4/1.5)	1.5 (1.4/1.6)	1.6 (1.4/1.7)	1.4 (1.3/1.5)
Diet Cl⁻	1.4 (1.3/1.5)	1.5 (1.4/1.6)	1.5 (1.4/1.7)	1.4 (1.3/1.4)
Diet K⁺	10.6 (10.1/11.2)	11.1 (10.3/11.8)	11.5 (10.5/12.6)	10.2 (9.6/10.9)
<i>Daily dietary electrolyte intake, average Week 1 to 10, mmol/day (95% C.I.)</i>				
Diet Na⁺	1.4 (1.4/1.4)	1.4 (1.4/1.5)	1.4 (1.4/1.5)	1.4 (1.3/1.5)
Diet Cl⁻	1.4 (1.3/1.4)	9.1 (8.7/9.5)	1.4 (1.3/1.4)	1.4 (1.3/1.4)
Diet K⁺	2.6 (2.5/2.6)	10.5 (10.1/11.0)	10.4 (10.0/10.8)	2.6 (2.5/2.7)

* HCTZ: Hydrochlorothiazide, 25 mg/kg BW per day, with diet; this dose of HCTZ was shown to attenuate the increase in BP and extend lifespan in the SHRSP (Gries J, Kretschmar R, Neumann BW. *J Cardiovasc Pharmacol.* 1989;13 (Suppl. 4):S79-82).

[†] Food intake did not vary throughout the study.

Table 2

Baseline blood pressure, body weight, creatinine and protein excretion

Variable	TZ	KCLTZ	KBCTZ	CTL
n	11	10	11	11
SBP, mmHg	178 (174/182)	174 (169/179)	176 (171/181)	175 (170/180)
Body weight, g	220 (214/226)	226 (215/237)	218 (208/228)	219 (208/230)
UCrV, mg/d	11 (10/12)	12 (11/13)	12 (10/14)	11 (10/12)
UCrV/100g BW	5.1 (4.8/5.4)	5.5 (5.3/5.7)	5.1 (4.8/5.4)	5.1 (4.9/5.3)
UPV, mg/d	66 (59/73)	75 (67/82)	72 (62/81)	64 (58/70)

UCrV: Urine creatinine excretion; BW: Body weight; UPV: Urine protein excretion.

Between group comparison: P=ns for all variables. Values are mean and 95% C.I.

Table 3

Urine volume and urinary electrolyte excretion

Group	TZ	KCLTZ	KBCTZ	CTL
<i>Urine Volume, mL/day (95% C.I.)</i>				
Wk 0	12.8 (11.4/13.4)	14.4 (12.6/16.2)	13.1 (11.2/14.9)	12.1 (11.0/13.3)
Average Day 1-3	9.7 (8.3/11.0) *	20.7 (18.0/23.4) *	17.6 (15.8/19.5) *	7.2 (6.5/8.0) *
Average Wk 4-10	9.9 (8.5/11.3)	22.2 (20.5/23.8) *	17.9 (15.6/20.2) *	12.6 (11.6/13.6)
<i>Baseline Na[±] balance mmol/day (95% C.I.)</i>				
Wk 0	0.04 (0.03/0.05)	0.03 (0.02/0.05)	0.05 (0.0/0.1)	0.03 (0.01/0.05)
<i>Urine Na[±] excretion, mmol/day (95% C.I.)</i>				
Wk 0	1.0 (0.8/1.2)	1.2 (1.0/1.3)	1.2 (1.0/1.4)	1.1 (0.9/1.2)
Average Day 1-3	1.3 (1.2/1.4) *	1.5 (1.4/1.7) *	1.4 (1.3/1.6) *	1.2 (1.0/1.3)
Average Wk 4-10	1.3 (1.2/1.5) *	1.8 (1.7/2.0) *	1.5 (1.4/1.5) *	1.4 (1.2/1.5) *
<i>Urine Cl⁻ excretion, mmol/day (95% C.I.)</i>				
Wk 0	1.5 (1.5/1.6)	1.6 (1.5/1.8)	1.7 (1.4/1.9)	1.6 (1.5/1.6)
Average Day 1-3	1.9 (1.8/2.0) *	8.8 (7.6/9.9) *	2.2 (2.0/2.4) *	1.7 (1.7/2.1)
Average Wk 4-10	1.8 (1.7/2.0) *	10.3 (9.1/11.6) *	2.2 (2.0/2.3) *	2.0 (1.8/2.2) *
<i>Urine K[±] excretion, mmol/day (95% C.I.)</i>				
Wk 0	9.0 (8.0/10.0)	10.0 (8.8/11.2)	9.2 (7.9/10.4)	8.8 (7.9/9.7)
Average Day 1-3	4.1 (3.9/4.3) *	12.2 (10.6/13.8) *	10.7 (9.7/11.7) *	3.8 (3.4/4.1) *
Average Wk 4-10	3.5 (3.2/3.8) *	13.3 (12.8/13.8) *	10.7(10.1/11.3)	3.6 (3.4/3.7) *

* Values are means and 95% C.I. Within group comparison: value differs from week 0, P<0.05.

Table 4

Heart rates (bpm) in SHRSP at baseline and during days 1 to 3 after assignment, 24-hour average

Day	TZ	KCLTZ			KBCTZ			CTL		
		mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	
0		402	392/412	390	383/398	403	392/414	383	376/391	
1	391 *	382/401	383	376/391	396	385/407	378	371/385		
2	387 *	380/395	382 *	375/388	393 *	382/405	377	370/384		
3	391 *	383/399	388	380/396	400	389/410	386	379/393		

* Indicates that heart rate is significantly lower compared to baseline, $P < 0.05$. Heart rate decreased by about 1.5% in KCLTZ and KBCTZ and by almost 3% in TZ. No change occurred in CTL.