Effect of Dietary Potassium Chloride in Borderline Hypertensive Rats¹ ²

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ABSTRACT
The borderline hypertensive rat is the first filial offspring of the spontaneously hypertensive rat and the Wistar-Kyoto rat. With increased dietary sodium chloride intake, the borderline hypertensive rat develops hypertension and exaggerated cardiovascular and renal responses to acute environmental stress, similar to those observed in the hypertensive spontaneously hypertensive rat parent. In other models of sodium chloride-sensitive hypertension with different genetic background (Dahl rat), dietary potassium chloride supplementation protects against the development of hypertension, increased sympathetic nervous system activity, and exaggerated responses to acute environmental stress. This investigation sought to determine whether the dietary sodium chloride-induced development of both the hypertension and the exaggerated responses to acute environmental stress could be reversed or prevented by increased dietary potassium chloride intake. Dietary potassium chloride intake was increased with a 1% potassium chloride drinking solution either after 12 wk of 8% sodium chloride intake (reversal) or concomitant with the onset of 12 wk of 8% sodium chloride intake (prevention). An increase in dietary potassium chloride intake did not reverse or prevent the development of either the hypertension or the exaggerated cardiovascular and renal responses to acute environmental stress in borderline hypertensive rats fed 8% sodium chloride. It is concluded that the difference in genetic background between borderline hypertensive rats and other models of sodium chloride-sensitive hypertension is an important determinant of the protective effect of dietary potassium chloride supplementation.

Key Words: Kidney, renal nerves, sodium, potassium, hypertension, environmental stress

The central nervous system and the kidneys have long been thought to be critical in the pathophysiology of hypertension. Folkow (1) has advanced the concept that primary hypertension derives from an interaction between genetic and environmental factors. Of the environmental factors, dietary NaCl intake and environmental (psychosocial) stress are considered important. Previous work (2–9) has defined an interaction between dietary NaCl intake and environmental stress in three experimental models characterized by genetic predisposition to hypertension, the spontaneously hypertensive rat (SHR), the Dahl rat, and the borderline hypertensive rat (BHR). Increased dietary NaCl intake increased arterial pressure and augmented the cardiovascular, effenter renal sympathetic nerve activity (ERSNA), and renal functional responses to acute environmental stress in these models.

The BHR is a genetic model of environmentally induced hypertension. The BHR is the first filial offspring of SHR and the normotensive Wistar-Kyoto (WKY) rat and possesses genetic information from both a normotensive WKY and a hypertensive SHR parent. As described by Lawler and colleagues (10–14), the BHR become permanently hypertensive when subjected to a time-limited period of exposure to environmental stress or to increased dietary sodium intake. Renal denervation, performed early but not late, can prevent the development of environmental stress–induced hypertension in BHR (15).

Increased dietary sodium intake causes the BHR to exhibit multiple characteristics of the phenotype of the hypertensive SHR parent. In addition to the development of sustained hypertension, BHR subjected to increased dietary sodium intake also manifest exaggerated natriuretic and renal sympathoinhibitory responses to iv isotonic saline loading (2), enhanced ERSNA and anti-natriuretic responses to environmental stress (2), and increased responsiveness of central nervous system α-2 adrenoceptors regulating ERSNA (16), each of which is similar to the response of the hypertensive SHR parent (2, 4, 17).
The BHR is a model of NaCl-sensitive hypertension. In other models of NaCl hypertension, DOCA-NaCl and Dahl NaCl-sensitive rats, dietary KCl supplementation has a protective effect against the dietary NaCl-induced development of increased mean arterial pressure (18–22) and increased sympathetic nervous system activity (20,21). In DOCA-NaCl rats, dietary KCl supplementation was equivalent to renal denervation in abolishing the antinatriuretic response to acute environmental stress (22), an intervention known to increase ERSNA (23). In addition, dietary KCl supplementation lowers arterial pressure in adult SHR with established hypertension (24), the hypertensive parent of the BHR. This study sought to determine the effect of dietary KCl supplementation on mean arterial pressure (MAP) and the cardiovascular, renal, and ERSNA responses to acute environmental stress in BHR consuming 8% NaCl.

METHODS

Animals

This study used male BHR that were the first-generation offspring of SHR females and WKY males purchased from Taconic Farms (Germantown, NY). The rats were weaned at 4 wk of age. Standard laboratory rat chow and tap water were available to all rats until the dietary regimens were instituted as described below. All animal experimentation was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals.

Anesthesia

The rats were anesthetized with methohexital (20 mg/kg ip) supplemented with 10 mg/kg iv as needed.

Procedures

Chronic Catheterization. The rats were instrumented with tygon catheters in the left carotid artery and jugular vein. The catheters were tunneled to the back of the neck, flushed and filled with heparinized saline (100 U/mL), and plugged with stainless steel pins. Through a suprapubic incision, a polyethylene urinary bladder catheter (PE-240), modified from that of Gellai and Valtin (25), was flanged and sutured into the urinary bladder and was exteriorized and secured by being sutured to the adjacent muscle, tissue, and skin.

Renal Sympathetic Nerve Activity Recording Electrode. The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope (magnification, ×25), a renal nerve branch from the aortocaval ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire electrode. Renal sympathetic nerve activity was amplified (×10,000 to ×50,000) and filtered (low, 30; high, 3,000 Hz) with a Grass P511 Bandpass Amplifier. The amplified and filtered signal was channeled to a Tektronix 5113 Oscilloscope and a Grass Model 7DA polygraph for visual evaluation, to an audio amplifier/loudspeaker (Grass Model Am 8 Audio Monitor) for auditory evaluation, and to a rectifying voltage integrator (Grass Model 7P10). The integrated voltage and renal neurogram signals were displayed on the Grass polygraph. The quality of the renal sympathetic nerve activity signal was assessed by its pulse synchronous rhythmicity and by examination of the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with an iv injection of norepinephrine (3 μg). The renal sympathetic nerve activity remaining after maximal inhibition after norepinephrine administration was similar to the background noise observed approximately 30 min postmortem; this value was subtracted from all experimental values of renal sympathetic nerve activity. When an optimal renal sympathetic nerve activity signal was observed, the recording electrode was fixed to the renal nerve branch with silicone cement (Wacker Sil-Gel 604; Wacker Chemie, Berlin, Germany). The electrode cable was then secured in position by being sutured to the abdominal trunk muscles. Finally, the electrode cable was exteriorized and the flank incision was closed in layers.

Experimental Protocol

Protocol 1 (Reversal). At 4 wk of age, all rats were individually housed and randomly assigned to one of four groups. Two groups received 1% NaCl diet, and the other two groups received 8% NaCl diet, both with tap water drinking fluid ad libitum, from 4 to 20 wk of age. At 16 wk of age, the tap water drinking fluid was replaced by 1% KCl drinking solution ad libitum for one of the 1% NaCl groups and one of the 8% NaCl groups. The remaining 1% NaCl and 8% NaCl groups continued with tap water drinking fluid ad libitum (2). At 20 wk of age, all rats underwent chronic catheterization and implantation of a renal sympathetic nerve activity recording electrode.

Protocol 2 (Prevention). At 4 wk of age, all rats were individually housed and randomly assigned to one of two groups. Both groups received 8% NaCl diet; one group received tap water drinking fluid ad libitum, and the other received 1% KCl drinking fluid ad libitum. At 16 wk of age, all rats underwent chronic catheterization and implantation of a renal sympathetic nerve activity recording electrode.

After allowing 24 h for recovery from surgery, rats were placed in rat holders, which permit forward and backward movement and the collection of steady-
state urines. An iv infusion (58 µL/min) of isotonic saline containing sufficient quantities of inulin and para-aminohippurate (PAH) for the determination of inulin and PAH clearance, respectively, was then started and allowed to continue throughout the duration of the experimental protocol. Four to 6 h after habituation and the start of isotonic saline infusion, the arterial catheter was flushed and attached to a pressure transducer and the urinary bladder catheter was lead to a collection beaker. After stabilization of urinary flow rate, arterial pressure, and heart rate, the quality of the renal sympathetic nerve activity recording was again tested with an iv injection of norepinephrine (3 µg) as previously described to ensure the absence of noise due to mechanical movement, respiration, or heart rate. If the quality of the renal sympathetic nerve activity recording was the same as that observed when the electrode was implanted, then the experiment commenced.

**Acute Environmental Stress.** The control period consisted of two consecutive 10-min urine collections. The acute environmental stress was applied for 25 min by air jet stress. Five minutes was allowed after the start of air jet stress before two consecutive 10-min urine specimens were collected (air jet stress period). Five minutes was allowed after the cessation of air jet stress before two consecutive 10-min urine specimens were collected (recovery period). Venous blood samples (200 µL) were taken during the mid-point of the control, air jet stress, and recovery periods. Acute environmental stress stimulation consisted of an air jet delivered to the top of the rat’s head through a tube located 4 to 5 cm in front of the rat. Repeated applications of air jet stress in the same rat result in a similar increase in heart rate, MAP, and ERSNA and in decreases in urinary flow rate and sodium excretion (2,3–7,8,16,17,23,26,27). The quality of the renal sympathetic nerve signals was again assessed with iv injections of norepinephrine (3 µg) after the completion of the protocol. The rat was then killed, and postmortem renal sympathetic nerve activity was continuously recorded for 30 min as a measure of background noise. The kidneys were removed, decapsulated, drained, and weighed.

### Analytical

Urinary volume was determined gravimetrically. Urine and plasma sodium and potassium concentrations were measured by flame photometry. Urine and plasma inulin and PAH concentrations were determined by the anthrone (28) and ethylenediamine methods (29), respectively. GFR was measured as inulin clearance: $C_{\text{in}} = \frac{V}{(U_{\text{in}})/P_{\text{in}}}$, where $U_{\text{in}}$ and $P_{\text{in}}$ are urine and plasma inulin concentrations, respectively, and $V$ is urine flow rate. Effective RPF was determined by PAH clearance: $(V).\frac{(U_{\text{PAH}})}{P_{\text{PAH}}}$, where $U_{\text{PAH}}$ and $P_{\text{PAH}}$ are urine and plasma PAH concentrations, respectively. Heart rate was determined with a linear cardiotachometer (Grass 7P4) driven by the arterial pressure waveform. Data acquisition was performed with a commercially available computer software package (Labetech Notebook 4.2; Marlborough, MA). Integrated renal sympathetic nerve activity is expressed as integrator resets/minute.

Statistical analysis was conducted with repeated-measures analysis of variance for main effects and interactions and Scheffé’s test for pairwise comparisons among means (30). A $P < 0.05$ was considered statistically significant in each case.

### RESULTS

#### Protocol 1 (Reversal)

The results are illustrated in Figures 1 through 4. The responses to acute environmental stress are summarized in Figures 1 and 2. The effects of the 4 wk of dietary KCl supplementation on basal values and the responses to stress are summarized in Figures 3 and 4, respectively, where they are compared with results from animals not receiving dietary KCl supplementation (2).

During the control period, heart rate (422 ± 11 versus 407 ± 13 beats/min), MAP (151 ± 4 versus 144 ± 4 mm Hg), and urinary sodium excretion (1.9 ± 0.4 versus 0.6 ± 0.1 µEq/min/g kidney wt) were significantly higher in 8% NaCl/1% KCl BHR than in 1% NaCl/1% KCl BHR, whereas urinary potassium excretion (1.7 ± 0.1 versus 1.8 ± 0.2 µEq/min/g kidney wt) was similar.

Acute environmental stress produced significant increases in heart rate, MAP, and urinary flow rate in both groups. The increases in heart rate and MAP were significantly greater in the 8% NaCl/1% KCl...
Figure 2. Urinary flow rate (V), sodium excretion (UnaV), potassium excretion (UKV), and ERSNA responses to air jet stress (A1, A2) in BHR receiving either 1% NaCl (left portion) or 8% NaCl (right portion) from wk 4 to 20 (16 wk) and 1% KCl from wk 16 to 20 (4 wk). CI, C2, control; R1, R2, recovery; KW, kidney weight.

Figure 3. Basal values for MAP, heart rate (HR), urinary flow rate (V), and sodium excretion (UnaV) in BHR receiving either 1% NaCl or 8% NaCl from wk 4 to 16 (12 wk) without KCl supplementation (left portion, from reference 2) and in BHR receiving either 1% NaCl or 8% NaCl from wk 4 to 20 (16 wk) and 1% KCl from wk 16 to 20 (4 wk, right portion). Abbreviations: bpm, beats per minute; KW, kidney weight.

Figure 4. Responses to air jet stress for ΔMAP, heart rate (ΔHR), urinary flow rate (ΔV), urinary sodium excretion (ΔUnaV), and ERSNA in BHR receiving either 1% NaCl or 8% NaCl from wk 4 to 16 (12 wk) without KCl supplementation (left portion, from reference 2) and in BHR receiving either 1% NaCl or 8% NaCl from wk 4 to 20 (16 wk) and 1% KCl from wk 16 to 20 (4 wk, right portion). Abbreviations: bpm, beats per minute; KW, kidney weight.

BHR than in the 1% NaCl/1% KCl BHR (29 ± 7 versus 15 ± 6 beats/min and 9 ± 2 versus 3 ± 1 mm Hg), whereas the increase in urinary flow rate was significantly greater in the 1% NaCl/1% KCl BHR than in the 8% NaCl/1% KCl BHR (20 ± 4 versus 7 ± 2 μL/min/g kidney wt). Urinary sodium excretion did not change in the 1% NaCl/1% KCl BHR (−0.1 ± 0.1 μEq/min/g kidney wt) but decreased significantly in the 8% NaCl/1% KCl BHR (−0.8 ± 0.1 μEq/min/g kidney wt). Urinary potassium excretion decreased similarly in 1% NaCl/1% KCl BHR (−0.6 ± 0.2 μEq/min/g kidney wt) and 8% NaCl/1% KCl BHR (−0.8 ± 0.2 μEq/min/g kidney wt). Acute environmental stress produced significant increases in ERSNA in both groups; the increases in ERSNA were greater in the 8% NaCl/1% KCl BHR than in the 1% NaCl/1% KCl BHR (64 ± 18 versus 26 ± 10%). GFR and RPF were not affected in either group.

The responses to acute environmental stress of the 1% and the 8% NaCl BHR without dietary KCl sup-
Implementation (concurrently studied under the identical experimental protocol and laboratory conditions) have been reported (2). Four weeks of 1% KCl supplementation did not alter the relationship between the baseline values of the 1% and the 8% NaCl BHR for several variables (Figure 3). Specifically, baseline values of heart rate and MAP were greater in the 8% than in the 1% NaCl BHR with or without dietary KCl supplementation. With respect to the responses to acute environmental stress (Figure 4), it is evident that 4 wk of 1% KCl dietary supplementation did not alter the relationship between the responses of the 1% and the 8% NaCl BHR for any measured variable. That is, the increases in heart rate, MAP, and ERSNA and the decreases in urinary sodium excretion were similarly greater in the 8% NaCl BHR than in the 1% NaCl BHR. Also, the increases in urinary flow rate were similarly greater in the 1% NaCl BHR than in the 8% NaCl BHR. The apparent effect of KCl to proportionally attenuate the heart rate responses in 1% and 8% NaCl BHR is unexplained.

Protocol 2 (Prevention)

The results are illustrated in Figures 5 through 8. The responses to acute environmental stress are summarized in Figures 5 and 6. The effect of concomitant dietary KCl supplementation on basal values and the responses to stress are summarized in Figures 7 and 8, respectively.

During the control period, heart rate, MAP, and renal function were similar in both groups of 8% NaCl BHR. Concomitant dietary KCl supplementation resulted in significantly higher control period values of urinary flow rate (9.6 ± 2.7 versus 5.5 ± 1.2 μL/min/g kidney wt) and urinary potassium (1.0 ± 0.1 versus 0.3 ± 0.1 μEq/min/g kidney wt) but not sodium excretion. Acute environmental stress produced significant and similar increases in heart rate and MAP in both groups. The increase in urinary flow rate was greater in 8% NaCl/1% KCl BHR than in 8% NaCl BHR (16.1 ± 0.9 versus 6.0 ± 0.8 μL/min/g kidney wt), whereas the increases in ERSNA (56 ± 16 versus 37 ± 5%) and the decreases in urinary sodium and potassium excretion were not significantly different between the two groups.

DISCUSSION

These studies have demonstrated that dietary KCl supplementation in BHR consuming 8% NaCl for 12 wk did not reverse or prevent the development of hypertension or the augmented cardiovascular, renal sympathoexcitatory, or renal functional responses to acute environmental stress.

BHR, when maintained on a 1% NaCl diet, do not exhibit an increase in arterial pressure or augmented responses to acute environmental stress (2); this pattern is identical to that of their normotensive WKY parent (2,4). In contrast, BHR given an 8% NaCl diet demonstrate an increase in arterial pressure in association with augmented responses to acute envi-
Environmental stress as manifested by greater increases in heart rate, MAP, and ERSNA and a greater decrease in sodium excretion (2). The antinatriuresis occurs without changes in GFR or RPF and is abolished by prior renal denervation (26); these results indicate that the antinatriuresis is due to a direct effect of increased ERSNA to increase net renal tubular sodium reabsorption. These responses occurring in BHR given an 8% NaCl diet are similar to those previously observed in their hypertensive SHR parent (4). The diuretic response of BHR to acute environmental stress is due to a decrease in plasma vasopressin concentration and may be inhibited by the provision of exogenous vasopressin or lesioning of the anteroventral portion of the third ventricle, a critical site of regulation of vasopressin secretion (26). Because both the development of hypertension and the augmented responses to acute environmental stress were elicited in BHR by an 8% but not a 1% NaCl diet, the results suggest that the increased dietary NaCl intake is able to induce or unmask these mechanisms that are genetically conveyed to the BHR by the hypertensive SHR parent in a latent form.

In our previous studies, we had observed that increased dietary NaCl intake for 6 wk did not increase MAP in BHR, whereas there was a significant increase in MAP in BHR after 12 wk of increased dietary NaCl intake (2). Thus, in the reversal portion of this study, the dietary KC1 supplementation was introduced after 12 wk of increased dietary NaCl intake in order to examine whether dietary KC1 supplementation could reverse the influences of preexisting increased dietary NaCl intake. Clearly, this was not the case. These results indicate that the mechanisms influenced by increased dietary NaCl intake, which lead to the development of hypertension and the exaggerated antinatriuretic and renal sympathoexcitatory responses to acute environmental stress, are not reversible by subsequent dietary KC1 supplementation.

Previous investigators using different models of NaCl-sensitive hypertension, DOCA-NaCl and Dahl NaCl-sensitive rats, have shown that dietary KC1 supplementation has a protective effect against the dietary NaCl-induced development of increased MAP.
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(18–22) and the increased sympathetic nervous system activity (20,21), as well as against the renal functional responses to acute environmental stress (22). However, in those studies (18–22), the dietary KCl supplementation was begun concurrently with the increase in dietary NaCl intake. In the DOCA-NaCl rat, the concurrent administration of 0.2, 0.5, or 1.0% KCl in the 1.0% NaCl drinking solution prevented the subsequent development of hypertension. This protective effect of dietary KCl supplementation was associated with enhanced urinary sodium excretion and a reduction to normal of the increased cumulative sodium retention and the exchangeable sodium and renal norepinephrine turnover (as an index of ERSNA) seen in the DOCA-NaCl rats not given KCl. In addition, dietary KCl supplementation was as effective as renal denervation in abolishing the antidiuretic and antinatriuretic responses to acute environmental stress in the DOCA-NaCl rat, an intervention known to increase ERSNA in that model (23). It was concluded that dietary KCl supplementation prevented the development of hypertension in DOCA-NaCl rats by decreasing ERSNA, which resulted in a natriuresis with the prevention of excess sodium retention. Similar conclusions have been derived from studies with dietary KCl supplementation concurrent with the increase in dietary NaCl intake in the Dahl NaCl-sensitive rat (19).

Although these findings suggested that beginning dietary KCl supplementation concurrently with the increase in dietary NaCl intake in BHR would prevent the development of hypertension and exaggerated responses to acute environmental stress, the experimental results presented here do not support this view. The level of arterial pressure and the responses to acute environmental stress were similar in BHR fed 8% NaCl for 12 wk whether or not they received concomitant dietary KCl supplementation. With respect to the effect of dietary KCl supplementation on ERSNA, the increment in ERSNA during acute environmental stress was not significantly attenuated in the group receiving dietary KCl supplementation. The effect of dietary KCl supplementation to reverse arterial pressure elevation has been examined in SHR. For example, when 14-wk SHR with established hypertension were provided with either tap water or 0.5% KCl solution to drink for 3 wk, those drinking tap water increased their MAP by 20 mm Hg compared with only 7 mm Hg for those drinking 0.5% KCl (24). This effect could not be attributed to changes in vasoactive hormones, renal sodium handling, vascular reactivity, baroreceptor function, or vascular angiotensin II receptor affinity or number.

This failure of dietary KCl supplementation to prevent the development of hypertension and exaggerated responses to acute environmental stress in the BHR model of NaCl-sensitive hypertension may relate to fundamental differences between the BHR and the DOCA-NaCl and Dahl NaCl-sensitive models. In the DOCA-NaCl and Dahl NaCl-sensitive models, the increase in arterial pressure induced by the increase in dietary NaCl intake is not fully sustained if the dietary NaCl intake is returned to a normal level. In the BHR model, the increase in arterial pressure induced by the increase in dietary NaCl intake or environmental stress is sustained even if the dietary NaCl intake is returned to a normal level or the environmental stress is stopped. It is possible that the genetic background of the BHR, which differs substantially from that of DOCA-NaCl and Dahl NaCl-sensitive rats, is responsible for this different pattern of initiation and maintenance of increased arterial pressure with increased dietary NaCl intake. Because the hypertensive parent of the BHR, the SHR, has lowered arterial pressure with dietary KCl supplementation, it may be that the BHR inherits a trait from the normotensive WKY parent that offsets this so that the arterial pressure of BHR is unresponsive to dietary KCl supplementation. This may have implications for the mechanisms involved wherein increased dietary NaCl intake results in increased ERSNA with increased arterial pressure and enhanced responsiveness of the ERSNA to acute environmental stress.

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