Am J Physiol Heart Circ Physiol
280: H1793–H1801, 2001.

Sodium-induced rise in blood pressure is suppressed by androgen receptor blockade

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Received 30 June 2000; accepted in final form 21 November 2000

Caplea, Ann, Darcie Seachrist, Gail Dunphy, and Daniel Ely. Sodium-induced rise in blood pressure is suppressed by androgen receptor blockade. *Am J Physiol Heart Circ Physiol* 280: H1793–H1801, 2001.—Our objective was to test the hypothesis that *1*) a high Na (HNa, 3%) diet would increase blood pressure (BP) in male Wistar-Kyoto (WKY) and spontaneously hypertensive Y chromosome (SHR/y) rat strains in a territorial colony; *2*) sympathetic nervous system (SNS) blockade using clonidine would lower BP on a HNa diet; and *3*) prepubertal androgen receptor blockade with flutamide would lower BP on a HNa diet. A 2 × 4 factorial design used rat strains (WKY, SHR/y) and treatment [0.3% normal Na (NNa), 3% HNa, HNa/clonidine, and HNa/flutamide]. BP increased in both strains on the HNa diet ($P < 0.0001$). There was no significant decrease in BP in either strain with clonidine treatment. Androgen receptor blockade with flutamide significantly decreased BP in both strains ($P < 0.0001$) and normalized BP in the SHR/y colony. Neither heart rate nor activity could explain these BP differences. In conclusion, a Na sensitivity was observed in both strains, which was reduced to normotensive values by androgen blockade but not by SNS blockade.

hypertension; testosterone; salt; territorial stress; clonidine; sympathetic nervous system; flutamide; kidney

IN HUMAN as well as in animal studies, males tend to have higher blood pressure (BP) (8, 31, 51, 61, 71) and are at greater risk for cardiovascular disease than premenopausal females (1, 3, 11, 43, 48, 55, 62, 64). Studies in which BP was manipulated by castration or treatment with sex hormones have implicated important cardiovascular effects of estrogen and testosterone (2, 8, 13, 29, 34, 35, 59, 72).

Our lab has shown that the Y chromosome from a spontaneously hypertensive rat (SHR) father when backcrossed into a normotensive Wistar-Kyoto (WKY) mother increased sympathetic nervous system (SNS) indexes (16) and maintained an increase in BP of about 15–20 mmHg even after 16 generations (16). The Y chromosome effect accelerated the pubertal rise of androgen levels (19) and required the androgen receptor for full effect (22). In addition, testosterone and the SHR Y chromosome (SHR/y) increased the storage and release of norepinephrine in the isolated kidney (37).

Dietary Na has also been implicated in hypertension (14, 27, 49, 54, 60). Although controversy still exists regarding the role of dietary Na in hypertension, certain species and many individuals within species are Na sensitive and experience significant changes in BP (12, 25, 50, 56, 69, 71). Using radiotelemetry for BP measurement, Calhoun et al. (7) found significant increases in both day and night mean arterial pressure (MAP) after high salt (HNa, 8% NaCl) in SHR and only night MAP for WKY rats. He argued that the WKY rat may be able to compensate for the increased Na load, whereas the SHR strain cannot.

In some cases, stress has been shown to potentiate the Na-induced rise in BP (42). The combination of a HNa diet and territorial stress, whereby animals socially interact and impose stress on one another, have been shown to increase BP (20, 23, 32). Because stress increases Na appetite (4, 18) it is not unusual for these to occur together and promote further hypertension.

BP measurement by radiotelemetry permits the continuous monitoring of cardiovascular parameters in freely moving, untethered animals. Such measurements can be analyzed for circadian BP, heart rate (HR), and activity (ACT) (66). Previous circadian rhythm studies of the SHR and the normotensive WKY rat have shown that BP, HR, and ACT are elevated during the dark cycle compared with the light cycle (28, 68). In these studies all experimental animals were individually housed. However, evidence from several labs suggests there are physiological differences in colony-housed animals compared with animals individually housed (23, 24, 33). For instance, housing animals under different environmental conditions has influenced behavioral, hormonal, immunological, and biochemical parameters (6, 5, 45, 53).

Therefore, there is a need to study cardiovascular regulation during daily life events to dissect the complex physiological processes involved (46). Our laboratory has focused on cardiovascular parameters in animals in social groupings and on the role of social rank on BP and neuroendocrine profiles (16, 21).

The following study tested the hypothesis that a HNa diet would elevate BP in animals with a SHR/y and WKY autosomes compared with normotensive

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WKY rats through a SNS and androgen mechanism. Therefore, our objectives were the following: *1*) to determine the effect of a HNa (3%) diet on BP in male WKY and SHR/y rats in a territorial colony environment; *2*) to determine whether SNS blockade using clonidine lowers BP on a HNa diet in a colony; *3*) to determine whether prepubertal androgen receptor blockade with flutamide lowers BP on a HNa diet; and *4*) to determine whether locomotor activity (ACT) or HR contributes to the enhanced BP effect.

METHODS

Rat Strains

Parental WKY/hsd and SHR/hsd strains were originally obtained from Harlan Sprague Dawley (Indianapolis, IN) and have been inbred in our laboratory since 1981. In the following studies we also used the consomic strain (SHR/y ua) developed in our lab, which has the SHR Y chromosome backcrossed into the WKY background for 17 generations (67). Briefly, a WKY female was mated with a SHR male. The males of the first generation were mated with a WKY female. This protocol was continued for 17 generations. As a result, 99.9% of the autosomes of the SHR/y strain are from the WKY strain and only the Y chromosome is from the SHR strain. Therefore, we compared the WKY and SHR/y in this experiment and differences in BP, HR, or ACT would implicate the Y chromosome because this is the only chromosome that is different between the two strains.

Rats were acclimated from birth to a 12-h light (0600–1800) -dark (1800–0600) cycle and this was continued throughout the entire experimental procedure with constant temperature (27–29°C) and humidity (50–70%). All animals were treated in a humane manner according to the National Institutes of Health guidelines and all experiments were approved by the University of Akron Institutional Animal Use and Care Committee.

Experimental Groups

Territorial colony. Each colony housed eight implanted male rats and eight female rats. The colony consisted of a large center box (1.23 m × 1.23 m × 15 cm) and four smaller (33 cm × 24 cm × 15 cm) side boxes containing food and water (15). Rats were free to move anywhere within the colony. Eight receivers were strategically placed under the center area and side nest boxes to ensure that cardiovascular and activity parameters could be monitored at all times (Fig. 1). Because of frequency overlap, only one rat could be monitored at a given time, all other radio transmitters were turned off. The data collected by all receivers were then used for the hourly average for that rat.

The experimental design included one WKY colony and one SHR/y colony of 12–14-wk-old rats maintained on normal food for 4 wk (NNa). Then 3% Na was added to the diet (HNa) for 6 wk and finally clonidine (120 μg/20 g food = 0.4 mg/kg body wt) was added to the 3% Na food (HNa/clonidine) for 6 wk. In addition, one WKY and one SHR/y colony were maintained on a HNa/flutamide (83 mg/kg body wt) diet beginning at 4 wk of age (while still in individual cages) and placed into colonies at 12 wk of age. They remained on the HNa/flutamide diet for 12 wk. The flutamide was then removed from the HNa/postflutamide diet for 4 wk and finally these colonies were placed on normal food (NNa/post-HNa/flutamide) for 4 wk. In summary, the experimental design consisted of two strains (WKY and SHR/y) and six treatments (NNa, HNa, HNa/clonidine, HNa/flutamide, HNa/postflutamide, NNa/post-HNa/flutamide). Because only one rat could be monitored in a colony at a time (details given in *Telemetry Equipment and Data Acquisition*), each rat was monitored once in a 2-wk interval. Therefore, each rat had at least two recordings for each different treatment. However, to ensure the drug treatment had reached its full effect, only the last single recording for each rat under each treatment contributed to our results.

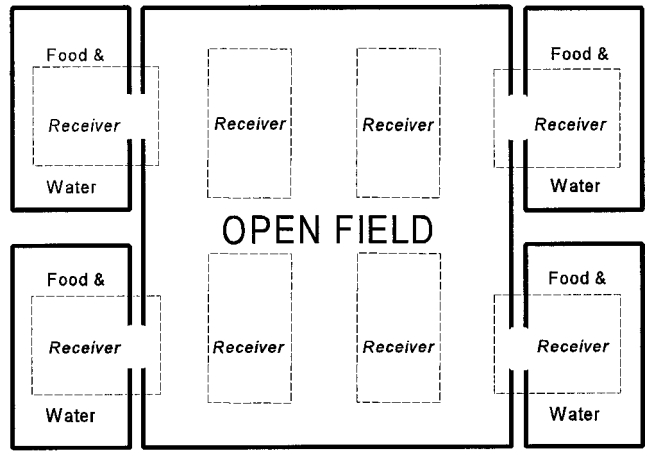

Fig. 1. Schematic of the colony housing. The colony consisted of a large center box (1.23 m × 1.23 m × 15 cm) and 4 smaller (33 cm × 24 cm × 15 cm) side boxes that contained food and water. Telemetry receivers were placed under the center and side boxes as shown.

Telemetry Equipment and Data Acquisition

Systolic BP, HR, and ACT were measured using a telemetry system and the Dataquest IV data-acquisition system (Data Sciences; Roseville, MN). Animals were anesthetized with brevital sodium (50 mg/kg ip, Eli Lilly; Indianapolis, IN) and the transmitters were surgically implanted. Briefly, a midline abdominal incision was made and the descending aorta was exposed between the renal vessels and the bifurcation of the femoral vessels. The vena cava and aorta were separated and a ligature was placed under the aorta to restrict blood flow caudally. A 21-gauge needle was used to make a small hole in the aorta and guide the flexible catheter tip of the radio transmitter into the aorta. The catheter was secured in place with a bonded patch (Vetbond, 3M Animal Care Products; St. Paul MN). The transmitter was placed in the peritoneal cavity and sutured to the abdominal wall as the midline incision was closed. Penicillin was administered (2,500 IU im) immediately after the surgery. Animals were placed into individual recovery cages for 1 wk.

Measurements of cardiovascular and activity parameters were recorded and saved every 30 min. Data were retrieved using the Sort Utility software from the Dataquest program. To illustrate the circadian pattern, sampling occurred once in a 30-min period (sampling time = 3 s). The data were then averaged to obtain a single value every hour for each of the 24 h in 1 day. The data represent a single day for each rat. Because of the design of the colony, only one transmitter could be "on" for a given day even though many rats with implants were housed in the colony. Each day the previously recorded rat was "turned off" and a new rat was "turned on." The three-way ANOVA worksheet included 24 data points of BP for each rat indexed by strain, housing, and time. Dark-cycle BP and HR were calculated as the average of the readings between 6 PM and 5 AM and the light-cycle BP and

HR as the average of the remaining readings for each rat. The three-way ANOVA worksheet included one dark and one light BP for each rat indexed by strain, housing, and cycle. Activity counts were obtained by monitoring changes in the signal strength that occurred as a result of movement of the transmitter. These data were used for statistics and to generate graphs.

Plasma samples were collected by retro-orbital puncture under brevital sodium anesthetic (50 mg/kg, Eli Lilly). A single sample per rat was collected at the end of each treatment period, just before any changes in treatment. Norepinephrine (NE) levels were assayed by HPLC with electrochemical detection (26). Testosterone levels were measured by RIA (Bio-Rad Laboratories). The correlation with another kit was $r = 0.991$, sensitivity was 0.08 ng/ml at the 95% confidence limit, and the highest cross-reactivity with potential interfering steroids was with 5 α-dihydrotestosterone (6.65%). The coefficient of variation for our sample range within run was 7.4% to 11.6% and for between run was 12.5% to 16.96%.

Statistics

Data were expressed as means ± SE. Differences among strain, treatment, and time were analyzed by three-way ANOVA. Comparisons of strain and time were analyzed by two-way ANOVA. Student's *t*-tests were used after ANOVA for pairwise comparisons. Significance was assumed if $P < 0.05$.

RESULTS

Our results demonstrated a significantly ($P < 0.001$) higher systolic BP in the SHR/y colony compared with the WKY colony on a NNa rat chow (Fig. 2). However, both the SHR/y (Fig. 3*A*) and WKY (Fig. 3*B*) colonies exhibited a significant ($P < 0.0001$) increase in BP on the HNa diet. Figure 4 shows a significant ($P < 0.001$) increase in plasma NE in both strains on a HNa diet. In addition, Fig. 4 shows that clonidine reduced NE levels in the SHR/y colony ($P = 0.007$), but an examination of Figs. 5 and 6 show that clonidine did not lower BP in either strain on a HNa diet. By contrast, flutamide significantly ($P < 0.0001$) reduced BP in both SHR/y (Fig. 5) and WKY colony rats (Fig. 6). A closer examination of the systolic BP by dark and light cycles (Fig. 7) revealed that although flutamide lowered BP in both strains on a HNa diet (SHR/y: 20 mmHg lower than HNa dark levels; WKY: 9 mmHg lower than HNa dark levels), it had its greatest effect in the SHR/y by lowering pressures to below the NNa BP level of the SHR/y [NNa dark (136 mmHg) compared with HNa/flutamide dark (134 mmHg)]. Flutamide had no effect on plasma NE levels (Fig. 4); however, it raised testosterone levels from an average of 1.04 to 10.0 ng/ml in the SHR/y colony and from 0.804 to 9.28 ng/ml in the WKY colony. Heart rates (Fig. 8*B*) were significantly lower in the WKY on the HNa/clonidine diet compared with HNa or HNa/flutamide, but there was no significant difference in HR between the HNa and HNa/flutamide diets. There was no significant difference in the SHR/y heart rates (Fig. 8*A*) in any of the treatments. Locomotor ACT (Fig. 9*B*) levels in the WKY decreased on the HNa/clonidine diet but there was no significant activity difference between the HNa and HNa/flutamide diets. SHR/y activity levels (Fig. 9*A*) were not significantly different among the treatment groups. Body weight did not significantly change with the increase in Na (Fig. 10, NNa vs. HNa) in either strain nor did it change with the reduction in dietary Na (Fig. 10, HNa/flutamide vs. NNa/post-HNa/flutamide). There was a significant strain difference ($P = 0.005$) in body weight between the HNa/flutamide colonies. Both strains showed a significant decrease in body weight (SHR/y, $P < 0.001$; WKY, $P = 0.002$) compared with the HNa and HNa/flutamide colonies.

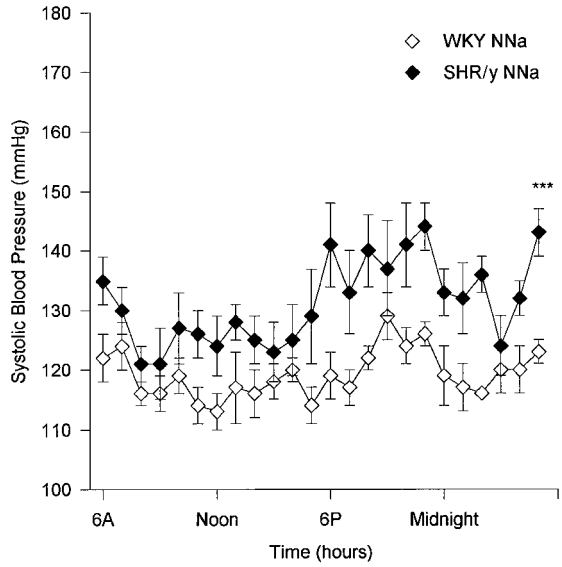

Fig. 2. Line graph of systolic blood pressure (SBP) in Wistar-Kyoto (WKY) and spontaneously hypertensive Y chromosome (SHR/y) colony rats. SBP expressed as means ± SE. There was a significant difference (three-way ANOVA) comparing strain (SHR/y and WKY: $P < 0.001$), treatment [normal Na (NNa), high NA (HNa), HNa/clonidine, and HNa/flutamide: $P < 0.001$] and time ($P < 0.001$). There was a significant interaction between strain and treatment ($P < 0.001$). A comparison of SHR/y and WKY colonies on a NNa diet shows a significant difference comparing strain with time [two-way ANOVA: strain degree of freedom (df) = 1, $F = 77.897$, $^{***}P < 0.001$; time df = 23, $F = 2.381$, $P < 0.001$]. There was not a significant interaction between strain and time. Twenty-four-hour average SBP for the SHR/y colony was 131 mmHg compared with 119 mmHg for the WKY colony. 6A, 6:00 AM; 6P, 6:00 PM.

DISCUSSION

Our data showed that the HNa/flutamide-treated colonies of both strains had lower systolic blood pressure (SBP) compared with HNa alone. In addition, when the flutamide was removed from the HNa/flutamide diet, SBP increased in a week suggesting there was not a permanent organizational effect on the cardiovascular control regions of the brain. However, plasma NE levels of both of the HNa/flutamide colonies were as high as that of the HNa colonies, yet BP was significantly lower.

Research has supported the role of the kidney in hypertension. A review by DiBona and Kopp (15) explores the complex involvement of physical, neu-

ral, and neuroendocrine regulation of kidney function. The activation of the SNS could contribute to renal hypertension via several mechanisms, including increased NE biosynthesis and release and/or through actions of angiotensin II, which can increase sympathetic nerve activity, Na retention, and BP.

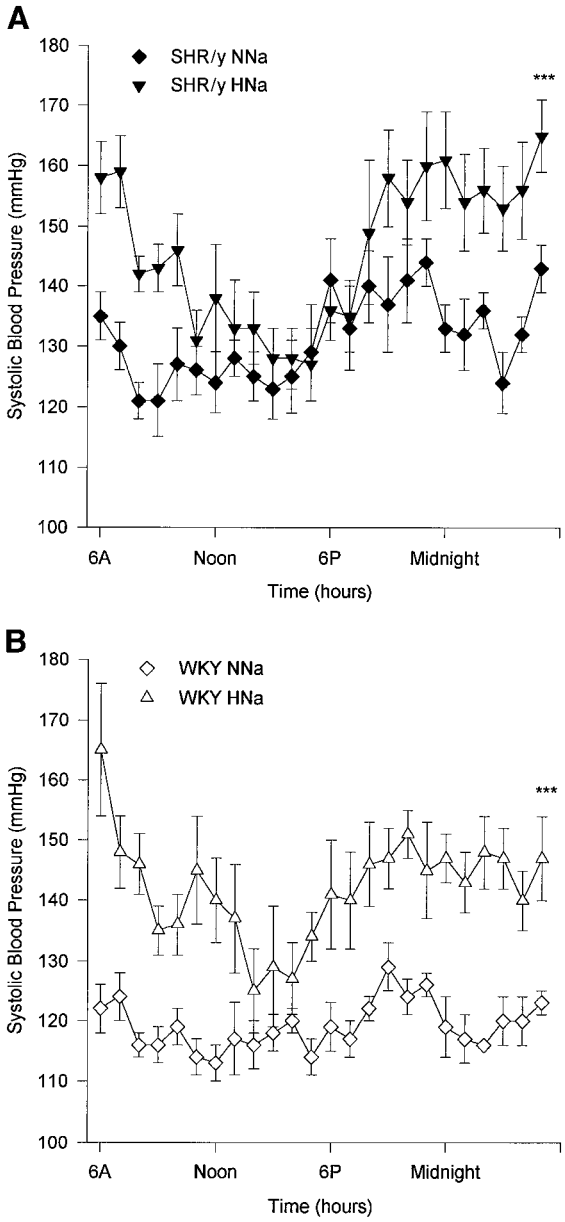

Fig. 3. *A*: line graph of SBP in the SHR/y colony rats on NNa and high Na (HNa) diets. SBP expressed as means ± SE. There was a significant difference in treatment compared with time (two-way ANOVA: treatment df = 1, $F = 68.56$, $^{***}P < 0.0001$; time df = 23, $F = 4.05$, $P < 0.0001$). There was no interaction between treatment and time. The average 24-h SBP for the NNa colony was 131 mmHg compared with 146 mmHg for the HNa colony. *B*: line graph of our SBP in the WKY colony rats on NNa and HNa diets. SBP expressed as means ± SE. There was a significant difference between treatment and time (two-way ANOVA: treatment df = 1, $F = 210.9$, $^{***}P < 0.0001$; time df = 23, $F = 2.15$, $P < 0.0023$). There was no interaction between treatment and time. The average 24-h SBP for the NNa colony was 119 mmHg compared with 142 mmHg for the HNa colony.

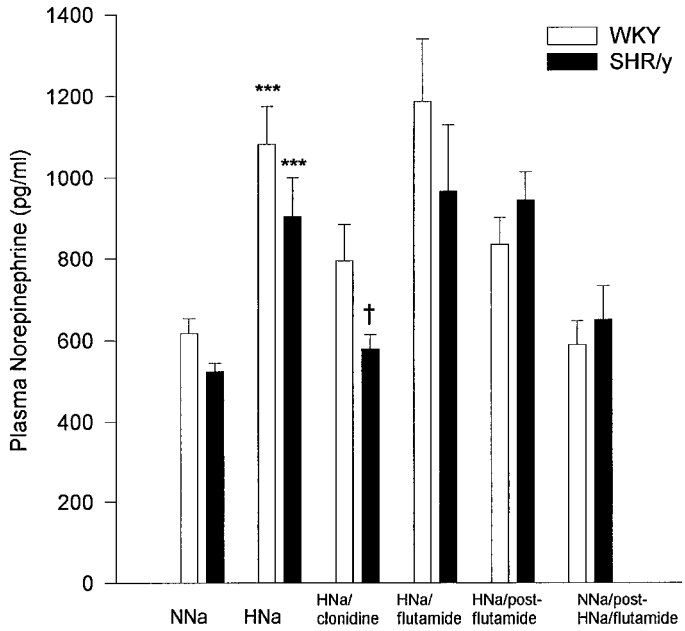

Fig. 4. Bar graph of plasma norepinephrine (NE)(pg/ml) levels in SHR/y and WKY colonies for 6 different treatments; NNa, HNa, HNa/clonidine, HNa/flutamide, HNa/postflutamide, and NNa/post-HNa/flutamide. NE values are expressed as means ± SE. There was no significant difference between the strains; however, there was a significant difference between treatments (two-way ANOVA: df = 5, $F = 10.172$, $P < 0.001$). There was no interaction between strains and treatments. NE levels significantly increased between the NNa and HNa diets (SHR/y: *t*-test $^{***}P < 0.001$; WKY: *t*-test $^{***}P < 0.001$). There was a significant decrease in NE levels in the SHR/y colony comparing HNa with HNa/clonidine ($^{\dagger}P = 0.007$) but this difference was not seen in the WKY colony.

Stimulation of renal sympathetic outflow may alter the normal relationship between arterial pressure and natriuresis. Reckelhoff et al. (57, 58) hypothesized that androgens increase arterial pressure in the SHR by causing a rightward shift in the pressure-natriuresis relationship, either by having the direct effect of increasing proximal tubular Na reabsorption or by activating of the renin-angiotensin system. Our lab has evidence that testosterone increases renal Na reabsorption and contributes to a rise in BP in SHR/y and WKY rats (70). Also ovarectomized SHR females with testosterone implants on a HNa diet had increased renal Na reabsorption compared with controls (44). Gong et al. (30) showed that renal α_2-adrenergic receptor density was higher in males than females in both SHR and WKY rats and castration of males reduced the renal α_2-adrenergic density by 50%, whereas testosterone treatment returned the receptor density to control levels. These findings support the hypothesis that increased SNS activation and testosterone may facilitate Na reabsorption in the kidney which can lead to an increased BP. Further studies in our lab are exploring this mechanism.

Another way flutamide lowered BP may be related to change in the social dynamics of the colony. In a colony, there are many social factors operating that can influence BP. We have previously shown (10) that nocturnal MAP increased in a colony compared with the individ-

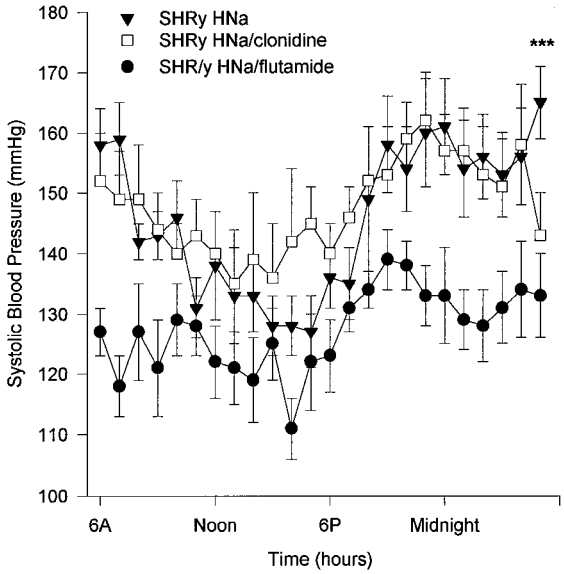

Fig. 5. Line graph of our SBP in the SHR/y colony rats on HNa), HNa/flutamide, and HNa/clonidine diets. SBP expressed as means ± SE. There was a significant difference comparing all three treatments (two-way ANOVA: treatment df = 2, $F = 18.922$, $P < 0.001$); however, there was no difference in time. There was a significant difference comparing treatment and time in the HNa and HNa/flutamide treatments (two-way ANOVA: treatment df = 1, $F = 99.47$, ***$P < 0.0001$; time df = 23, $F = 3.62$, $P < 0.0001$). There was no significant difference comparing the HNa with HNa/clonidine treatments (two-way ANOVA: treatment df = 1, $F = 4.808$, $P < 0.0291$); however, there was a significance difference in time df = 23, $F = 4.634$, $P < 0.0001$). There was no interaction between treatment and time with any group. The average 24-h SBP for the HNa colony was 146 mmHg compared with 127 mmHg for the HNa/flutamide colony and 151 mmHg for the HNa/clonidine colony.

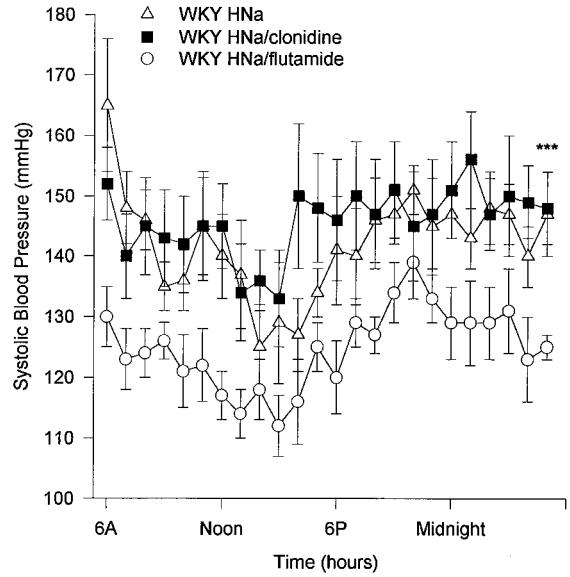

Fig. 6. Line graph of SBP in the WKY colony rats on HNa, HNa/flutamide, and HNa/clonidine diets. SBP expressed as means ± SE. There was a significant difference comparing all three treatments (two-way ANOVA: treatment df = 2, $F = 43.319$, $P < 0.001$); however, there was no difference in time. There was a significant difference comparing treatment with time in the HNa and HNa/flutamide treatments (two-way ANOVA: treatment df = 1, $F = 41.63$, ***$P < 0.0001$; time df = 23, $F = 2.575$, $P < 0.0002$). There was no significant difference comparing the HNa and HNa/clonidine treatments (two-way ANOVA: treatment df = 1, $F = 0.0035$, $P < 0.95$; time df = 23, $F = 1.51$, $P < 0.07$). There was no interaction between treatment and time with any group. The average 24-h SBP for the HNa colony was 142 mmHg compared with 127 mmHg for the HNa/flutamide colony and 141 mmHg for the HNa/clonidine colony.

ually caged rats (SHR/y 9.5 mmHg; WKY 3.2 mmHg). Flutamide treatment changed the social dynamics of both the WKY and SHR/y colonies. The social hierarchy usually present in the other colonies (16) was not observed in the flutamide-treated colonies. There was no evidence of aggressive behavior between the males as measured by scarring on their nose and backside. In addition, usually if an intruder was placed in the colony, the dominant rat defended the colony. This behavior was not observed in the flutamide-treated colonies probably due to the lack of the dominant-subordinate hierarchy. Evidence that flutamide altered reproductive behavior was the lack of any litters in the colonies. When flutamide was discontinued and testosterone levels normalized, conception occurred and numerous litters followed.

Our results demonstrated a significant Na sensitivity in the WKY. Most previous comparisons of WKY and SHR Na sensitivity were in low-stressed (individually caged) animals. When we compared caged WKY and SHR/y animals, our data did not show a Na sensitivity in the WKY rats. We have previously reported that the SHR strain but not the WKY strain was Na sensitive in combination with the high stress of the territorial colony (20, 23). However, our previously published data were not recorded by telemetry, but by tail-cuff, so the data were not previously recording a

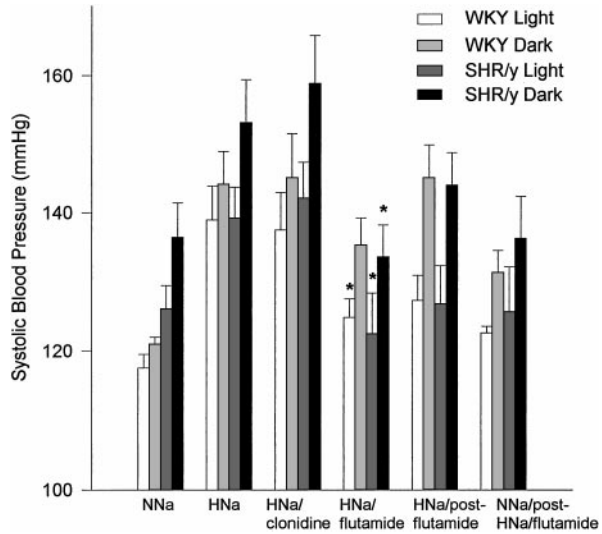

Fig. 7. Bar graph of a 12-h average SBP comparing light and dark cycles of WKY and SHR/y rats on six different diets; NNa, HNa, HNa/clonidine, HNa/flutamide, HNa/postflutamide, and NNa/post-HNa/flutamide. SBP expressed as means ± SE. There was a significant difference comparing treatments (one-way ANOVA: df = 11, $F = 5.71$, $P < 0.001$). Flutamide lowered BP in both strains (SHR/y HNa dark vs. HNa/flutamide dark: *$P = 0.035$; SHR/y HNa light vs. HNa/flutamide light: *$P = 0.042$; WKY HNa light vs. HNa/flutamide light: *$P = 0.034$). There was no significant difference between NNa and HNa/flutamide in both the light and dark cycles of the SHR/y colonies.

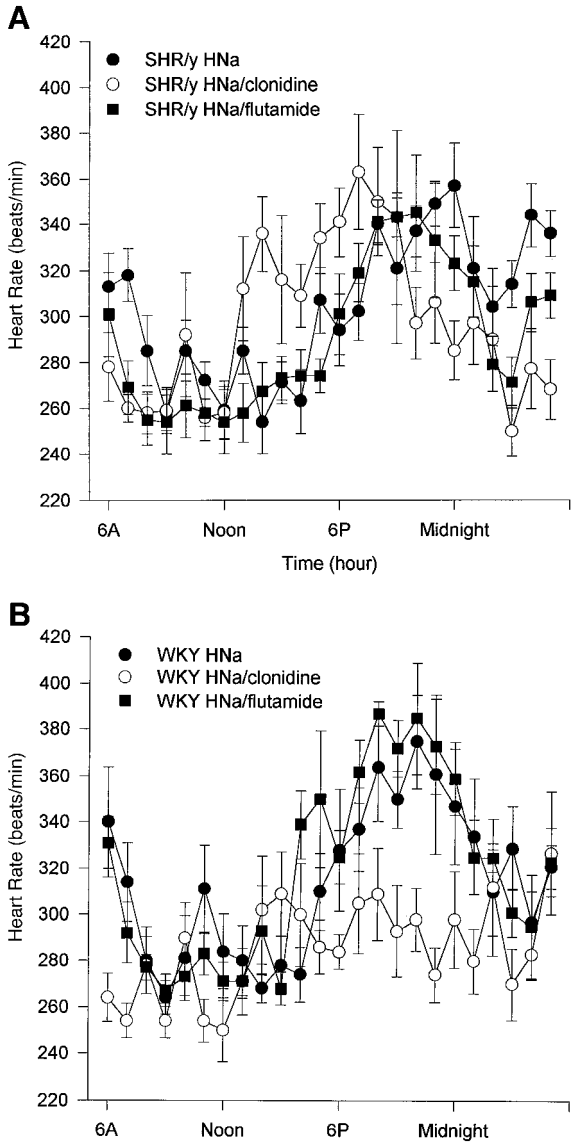

Fig. 8. *A*: line graph of the heart rate (HR) of SHR/y rats comparing HNa, HNa/clonidine, and HNa/flutamide diets. HR expressed as means ± SE. There was no significant difference in HR among the different diets. *B*: line graph of the HR of WKY rats comparing HNa, HNa/clonidine, and HNa/flutamide diets. HR expressed as means ± SE. There was a significant difference in HR among the treatment groups (two-way ANOVA; df = 2, $F = 15.527$, $P < 0.001$) and time (two-way ANOVA; df = 23, $F = 4.202$, $P < 0.001$). There was no interaction between treatment and time. There was a significant difference in the HNa/clonidine and HNa (*t*-test $^*P < 0.001$) and HNa/flutamide (*t*-test $^*P < 0.001$). There was no significant difference between the HNa and HNa/flutamide groups.

24-h range of pressures or comparing light and dark cycles. When we compared caged WKY and SHR/y animals by using radiotelemetry our data indicated that the WKY was only Na sensitive during the active dark but not the light, as corroborated by Calhoun et al. (7). Therefore, time of data collection could influence the results. Another reason for differences between laboratory results could be the differences between the strains. Our WKY and SHR strains were originally obtained by Harlan Sprague Dawley and inbred in our lab since 1981. Because we have not selected rats for specific BP traits while breeding it is possible that over generations the BP of the WKY and SHR strains may migrate toward each other (personal communication, Harlan Sprague Dawley). This is possible because the SHR strain originated from WKY and was selected for hypertension. We have also shown (35) that the WKY strain is polymorphic and was not an inbred strain before it was outbred.

Our results showed a significant increase in plasma NE levels in both strains on the HNa diet compared with the NNa. In addition, there was a significant

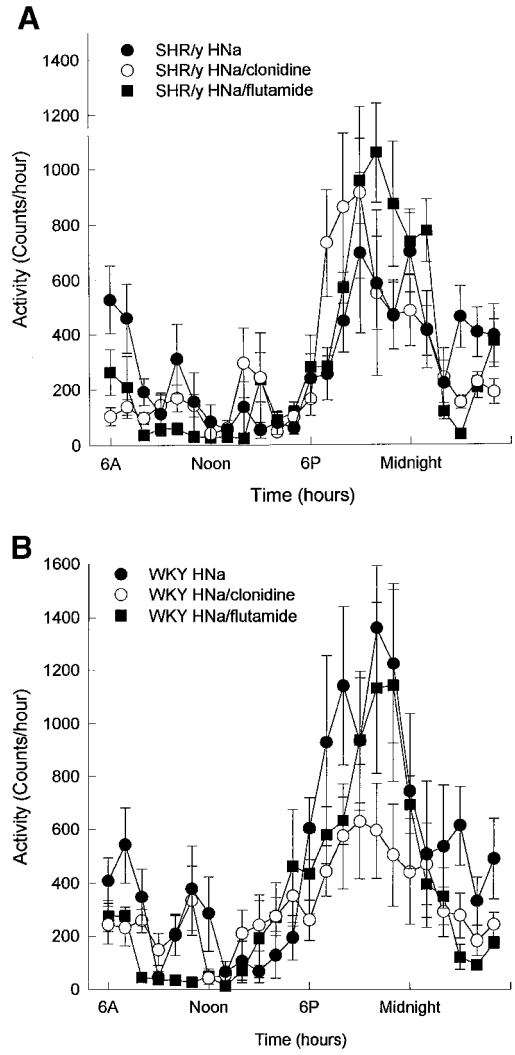

Fig. 9. *A*: line graph of activity (ACT) levels of SHR/y comparing HNa, HNa/clonidine, and HNa/flutamide diets. ACT expressed as means ± SE. There was no significant difference among the different treatments of the SHR/y colonies. There was a significant difference in time (two-way ANOVA; df = 23, $F = 7.038$, $P < 0.001$). There was no interaction between treatment and time. *B*: line graph of the ACT levels of WKY rats comparing HNa, HNa/clonidine, and HNa/flutamide diets. ACT is expressed as means ± SE. There was a significant difference in treatment and time for the three treatments (two-way ANOVA: df = 2, $F = 10.035$, $P < 0.001$; time: df = 23, $F = 9.975$, $P < 0.001$). There was no interaction between treatment and time. The HNa/clonidine levels were significantly lower than the HNa treatment ($P < 0.001$).

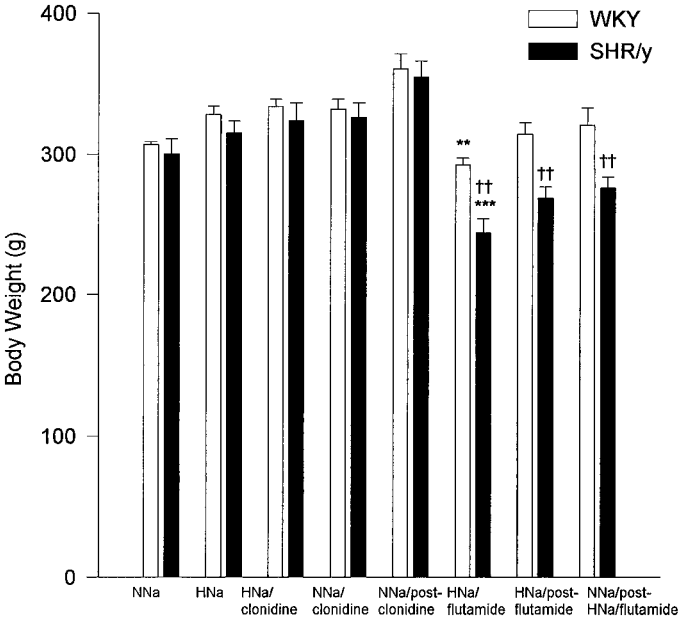

Fig. 10. Bar graph showing the effects of average body weight for both SHR/y and WKY colonies on 8 different treatments: NNa, HNa, HNa/clonidine, NNa/clonidine, NNa/postclonidine, HNa/flutamide, HNa/postflutamide, and NNa/post-HNa/flutamide. There was a significant difference among the treatment groups (one-way ANOVA; df = 15, $F = 10.108$, $P < 0.001$). There was no significant difference comparing the HNa/clonidine and the NNa/clonidine in both strains. There was no significant difference between the HNa/flutamide and the NNa/flutamide in either strain. There was a significant decrease comparing HNa vs. HNa/flutamide in both strains (SHR/y: ***$P <$ 0.001; WKY: **$P = 0.002$). There was no significant difference by strain on the NNa, HNa, HNa/clonidine, or NNa/postclonidine treatments. There was a significant strain difference with flutamide treatment (HNa/flutamide: ‡$P = 0.005$; HNa/postflutamide ‡$P =$ 0.005; and NNa/post-HNa/flutamide: ‡$P = 0.009$).

increase in BP in both strains on the HNa diet. Many previous studies have demonstrated a similar relationship between a HNa intake and increased SNS activation (9, 38, 65).

It appears that neither HR nor ACT can explain the increase in BP with increased dietary Na because there was no significant HR or ACT difference between the HNa and HNa/flutamide treatments in either strain yet BP was significantly greater in the HNa colonies. In addition, although BP remained elevated in both strains during all dark hours from 6 PM to 6 AM on the HNa diet, HR reached a peak at midnight for the SHR/y rats and around 10 PM for the WKY animals and then declined in both strains. A similar pattern was established with ACT, which peaked at 9 PM for the SHR/y rats and 10 PM for the WKY animals but was not constantly elevated throughout the dark hours like BP.

As previously mentioned, our results showed a significant increase in plasma NE levels and BP in both strains on the HNa diet compared with the NNa diet. However, when clonidine was added to the diet, NE levels decreased but BP did not significantly decrease. Previously (10), we reported a significant BP decrease in the SHR/y (but not the WKY) rats when clonidine was added to the HNa diet. However, in that study, animals were on a moderately HNa diet of 3% NaCl, which is 1.2% Na compared with this study which is 8% NaCl or 3% Na. In addition, the BP values were obtained through weekly tail-cuff measurements. In the previous (10) and our current study, the clonidine dosage was based on a prior study in which we found that 120 μg/20 g food could reduce BP in a caged animal on a moderately HNa diet (1.2% Na). In support of this dosage, in these same animals, when the HNa was removed from the food but the clonidine was still present, BP dropped below that of the NNa levels. So it appears that the clonidine dosage was sufficient to attenuate BP but not when the dietary Na levels were elevated from 1.2% to 3% Na. It does not appear that the decrease in BP is a result of a blood volume decrease because body weight did not change with the reduction in Na in the diet (Fig. 10). Previous studies of HNa (3% Na) and fluid volume indicators showed no long-term changes in cardiac output, central blood volume, hematocrit, total body water, or plasma Na between SHR on a control Na (12 mmol/100 g food = 0.3% Na), low Na (0.5 mmol/100 g food = 0.03% Na), or HNa (120 mmol/100 g food = 3% Na) diet (17). There was, however, a significant decrease in body weight in both strains on the flutamide diets compared with controls. Because testosterone is an anabolic steroid the reduced body weight is probably a result of the androgen receptor blockade during growth and development because the flutamide treatment began at 4 wk of age. We also noted a significantly lower body weight in the flutamide-treated SHR/y compared with the WKY strain. Because it had been previously reported that the SHR/y strain produces an earlier testosterone rise than the WKY strain (19), it also appears that the SHR/y rats may be more sensitive to androgen receptor blockade and more dependent on testosterone for body weight.

Another interesting comparison showed that there was no significant difference between the plasma NE levels of the HNa/flutamide colonies compared with HNa alone, yet BP was significantly lower in the HNa/flutamide colonies. It is possible that other indexes (i.e., recordings, tissue NE, 24-h urine) may be a better indicator of SNS activity than plasma NE. However, a more influential mechanism responsible for the increased BP may be that salt sensitivity is mediated through the SNS but potentiated by testosterone. This relationship may be mediated through a mutual effect on NE because testosterone influences NE metabolism, storage, and release (41). We have also shown that renal fractional release of NE (amount released per unit of time per total content of the organ) is enhanced by testosterone (37). Therefore, there is a link between testosterone enhancing NE release, which could also increase Na reabsorption through known SNS mechanisms in the kidney and gastrointestinal tract (63) Also, castration decreases the density of adrenergic neurons and produces morphological changes that are reversed by testosterone replacement (52).

Further evidence supporting an interaction of testosterone and NE is shown by Kumai et al. (40) who demonstrated that epinephrine and NE levels, tyrosine hydroxylase (TH) activity, and TH mRNA in the adrenal medulla of SHR was potentiated by testosterone and the TH mRNA expression in the adrenal medulla of SHR was higher than that of WKY rats. In addition, the affinity of the androgen receptor but not its density was higher in the SHR adrenal medulla compared with that of WKY (39). Similarly, McConnaughey and Iams (47) showed that androgens modulate the number of α_1-adrenoceptors in blood vessels of the male SHR.

In conclusion, both the WKY and SHR/y colonies exhibited significantly higher systolic BP on a HNa (3% Na) diet compared with a NNa (0.3%) diet. Although the SHR/y colony had higher BP than the WKY colony, there was a similar Na sensitivity observed in both strains. In each diet, the treatment-matched SHR/y strain had significantly higher BP than the WKY strain except when the androgen receptor was blocked by flutamide. SNS blockade with clonidine was not able to lower BP when combined with a HNa diet even though plasma NE levels were reduced. The BP differences among strains and treatments could not be explained by HR or ACT differences. The most significant observation of this study was that prepubertal androgen receptor blockade reduced the Na-induced rise in SBP of both WKY and SHR/y colonies to values within the normotensive range.

The authors express appreciation for the technical support of Fieke Bryson and Sarah Francis.

This research was supported by National Heart, Lung, and Blood Institute Grants HL-48072-07, and by the Ohio Board of Regents Grants to the Hypertension Center, University of Akron.

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