Circadian rhythm of plasma sodium is disrupted in spontaneously hypertensive rats fed a high-NaCl diet

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Fang, Zhiwu, Scott H. Carlson, Ning Peng, and J. Michael Wyss. Circadian rhythm of plasma sodium is disrupted in spontaneously hypertensive rats fed a high-NaCl diet. Am J Physiol Regulatory Integrative Comp Physiol 278: R1490–R1495, 2000.—High-NaCl diets elevate arterial pressure in NaCl-sensitive individuals, and increases in plasma sodium may trigger this effect. The present study tests the hypotheses that 1) plasma sodium displays a circadian rhythm in rats, 2) the plasma sodium rhythm is disturbed in spontaneously hypertensive rats (SHR), and 3) excess dietary NaCl elevates plasma sodium concentration in SHR. The results demonstrate that plasma sodium has a circadian rhythm that is inversely related to the circadian rhythm of arterial pressure. Although the plasma sodium rhythms of SHR and control rats are nearly identical, the plasma sodium concentrations are significantly higher in SHR throughout the 24-h cycle. Maintenance on a high-NaCl diet increases plasma sodium concentration similarly in both SHR and control rats, but it blunts the plasma sodium rhythm only in SHR. These results demonstrate that in rats, plasma sodium has a circadian rhythm and that high-NaCl diets increase plasma sodium concentration.

HYPERTENSION GREATLY INCREASES the risk of cardiovascular disease, including coronary heart disease, stroke, atherosclerosis, and end organ damage. In many individuals, a high-NaCl diet increases arterial pressure and can exacerbate this damage, but the mechanism linking NaCl intake to hypertension remains elusive. Several lines of evidence suggest that a high-NaCl diet increases arterial pressure primarily by increasing blood volume (11, 32), but other studies suggest that a high-NaCl diet may increase plasma sodium concentration, thereby activating central osmosensitive nuclei and reflexly increasing arterial pressure (3, 23, 24). Clinical studies have not found a close relationship between the amount of sodium in the diet and plasma sodium concentrations, but these studies have not considered the potential circadian variation in plasma sodium concentration. If such variations are large, the timing of blood sampling would be critical in studies of plasma sodium concentration.

Several rodent models have been developed to examine the mechanisms underlying salt-sensitive hypertension, e.g., spontaneously hypertensive rats (SHR), Dahl salt-sensitive rats, DOCA-NaCl treated rats, and transgenic high renin (mRen) rats (37, 41). In several of these models renal dysfunction contributes importantly to hypertension, but experimental evidence indicates that overactivity of the sympathetic nervous system also contributes to dietary NaCl-sensitive hypertension (37, 41, 50, 52). Several nuclei in the brain, e.g., the organum vasculosum of the lamina terminalis (3, 22, 23) and the subfornical organ (2, 3), respond to relatively small increases in circulating sodium by reflexly increasing sympathetic nervous system activity, vasopressin release, and drinking (3, 23, 45). If a high-NaCl diet increases plasma sodium, it could thereby increase sympathetic nervous system activity and arterial pressure. SHR display dietary NaCl-sensitive hypertension that has been postulated to result from excess sodium retention after exposure to a high-salt diet (13, 15, 48). Only a few studies have analyzed plasma sodium concentration, and none have considered whether plasma sodium levels fluctuate over a 24-h period.

Many biological systems display circadian rhythms that are linked to light-dark cycles. In normotensive Wistar-Kyoto rats (WKY) and SHR, mean arterial pressure (MAP) and heart rate (HR) display circadian rhythms (4, 6, 8), and the MAP rhythmic pattern is modified by changes in dietary NaCl in both strains (5, 8, 14). The current studies test three hypotheses: 1) that plasma sodium concentration displays a 24-h rhythm parallel to the MAP rhythm and that the plasma sodium rhythm contributes importantly to changes in MAP observed throughout the 24-h cycle; 2) that the circadian rhythm of plasma sodium is disrupted in SHR fed a basal NaCl diet, potentially contributing to hypertension in SHR; and 3) that a high-NaCl diet further disrupts the plasma sodium rhythm, which may lead to inappropriately elevated plasma sodium levels and increases in MAP in SHR.

MATERIALS AND METHODS

Male WKY and SHR (Harlan Sprague Dawley, Indianapolis, IN) were housed in individual cages in a sound-attenuated room at constant humidity (60 ± 5%), temperature (24 ± 1°C), and 12:12-h light-dark cycle (lights on at 0600–1800) throughout the experiments. All rats were allowed ad libitum access to basal (0.6% diet #8746, Teklad, Madison, WI) or high (diet #5008, Teklad)-NaCl diet and to tap water. All...
studies were approved by the University of Alabama at Birmingham's Institutional Animal Use and Care Committee and conducted in accordance with the regulations of the National Institutes of Health.

Surgical Procedures

At 9 wk of age, all rats were anesthetized with pentobarbital sodium (55 mg/kg body wt ip) and chronically instrumented with an indwelling Silastic arterial catheter that was advanced into the abdominal aorta via the left femoral artery. All catheters were tunneled subcutaneously to the midscapular region, where they were exteriorized and secured with dental acrylic. On recovery from anesthesia, rats were returned to their home cage and recovered for 3 days before the experiments were initiated. The arterial catheters were flushed daily with 100 µl of heparinized saline (20 U/ml). Experiments were initiated. The arterial catheters were flushed with 100 µl of saline (20 U/ml) and infused into the rat. For nighttime (1800–0600) sampling, a red light was used to avoid exposing the rats to light cues that might disrupt circadian rhythms. Only one blood sample was taken from a given animal each day (i.e., there were at least 26 h between samplings in a given rat) to prevent any significant change in hematocrit during the experimental period. Blood samples were taken at a randomly assigned hour on each day for 24 days, so that at the conclusion of the experiment there was a total of eight samples (1 per rat) for each 24 h.

After the 24-h plasma sodium concentration rhythm was characterized in WKY, the experimental protocol was repeated in SHR (n = 8); however, samples were taken at 2-h intervals instead of 1-h intervals (i.e., samples were collected for 12 time points).

Experiment 2: circadian rhythm of plasma sodium concentration on high (8%)-NaCl diet. In the second experiment, WKY (n = 32) and SHR (n = 32) were divided into four groups (8 rats/group), and each group was assigned to one of four different time points. These time points (0400, 0800, 1400, and 2000) were chosen on the basis of the nadir (0400) and the peak (1400) of the plasma sodium rhythm and one time point during which plasma sodium concentration was increasing (0800) and one time point during which plasma sodium concentration was declining (2000). Blood samples were drawn, as described above, at the assigned time points for 2 days while the rats were fed a basal (0.6%) NaCl diet. Rats were then placed on a high (8%)-NaCl diet, and blood samples were drawn at the same time points on days 4 and 5 after initiation of the high-NaCl diet.

MAP and HR. To assess whether the circadian plasma sodium rhythm correlated with the MAP and HR rhythms, simultaneous with the above experiments, parallel groups of WKY (n = 10) and SHR (n = 10) were implanted with telemetry probes for continuous monitoring of arterial pressure and HR, as described previously (8). WKY and SHR were divided into two groups, with one group receiving a basal (0.6%) NaCl diet and the second group receiving a high (8%)-NaCl diet. The data collection methods and techniques for analyzing MAP and HR circadian rhythms are described elsewhere (8).

Analysis of blood samples. The plasma was stored at −20°C for subsequent analysis of plasma sodium concentrations. Plasma sodium levels were measured using flame photometry (Allied Instrumentation Laboratory, Lexington, MA), and each sample was assayed three times during the analyses of that day’s samples. The flame photometer was recalibrated after every four samples, which resulted in a calculated intra-assay variation of ±0.2 meq/l. Hematocrit of all blood samples was measured using the microcapillary technique.

Statistical analysis. Within-group and between-group differences in plasma sodium were tested using ANOVA followed by post hoc Newman-Keuls analysis to determine the source of significant main effects or interactions. In all cases, a value of P < 0.05 was considered statistically significant. Results are presented as means ± SE.

RESULTS

Experiment 1

The 24-h plasma sodium concentration in WKY exhibited a peak that occurred during the daytime period (1200–1600; Fig. 1) with a plasma sodium concentration of 142.8 ± 0.3 meq/l (Fig. 1). The nadir of the rhythm occurred at night (0400) when plasma sodium concentration was 138.4 ± 0.2 meq/l. The circadian sodium rhythm in SHR was similar to that in

![Fig. 1. The 24-h circadian rhythm of plasma sodium concentration in Wistar-Kyoto (WKY; A; n = 8) and spontaneously hypertensive rats (SHR; B; n = 8) that are on basal NaCl diets. Time reflects clock hours, with lights on at 0600 and off at 1800. *P < 0.05 compared with previous time point.](http://ajpregu.physiology.org/10.1152/ajpregu.00003.2017)
WKY (Fig. 1). The peak of the rhythm occurred at 1400 (144.8 ± 0.3 meq/l), and the nadir of plasma sodium concentration occurred at 0400 (140.3 ± 0.4 meq/l). Plasma sodium concentrations in SHR were significantly higher than those of WKY at almost every time point measured (Fig. 2), with the greatest differences occurring at 2200 (142.2 ± 0.2 and 138.7 ± 0.3 meq/l; Fig. 2).

In SHR and WKY, the plasma sodium concentration and MAP rhythms were in nearly opposite phases (Fig. 3). In WKY, MAP peaked at 0400 and had a nadir during the daytime (Fig. 3). Thus the peak plasma sodium concentration occurs close to the MAP nadir, whereas the plasma sodium concentration nadir occurs near the MAP peak. In SHR, peak MAP also occurred at night (0400), whereas the nadir for MAP occurred at the end of the daylight period (Fig. 3).

**Experiment 2**

The effects of high NaCl on the 24-h plasma sodium rhythm in WKY and SHR were analyzed. On the basis of the results of experiment 1, plasma sodium concentration was examined at four time points (0400, 0800, 1400, and 2000). During exposure to the basal NaCl diet, both WKY and SHR displayed similar plasma sodium rhythms as those obtained in experiment 1, and, as in that experiment, circulating sodium levels were significantly elevated in SHR compared with WKY at all points (Fig. 4). In WKY, a 4-day exposure to the high-NaCl diet significantly increased plasma sodium concentration at all four time points, but did not appreciably alter the plasma sodium rhythm. In SHR, the high-NaCl diet also elevated the plasma sodium concentration at all time points, but it greatly blunted the plasma sodium circadian rhythm, i.e., there was no significant decrease in plasma sodium concentration between 1400 and 2000 and a blunted decrease at 0400 (Fig. 4).

In WKY fed the high-NaCl diet, peak MAP occurred at 0400, whereas the nadir MAP was at 1400 (Fig. 5). The MAP and plasma sodium rhythms were essentially opposite in WKY. In SHR on the high-NaCl diet, peak MAP occurred at 0400 and nadir MAP at 1400 (Fig. 5), and the plasma sodium rhythm was inversely related to the MAP rhythm.

**DISCUSSION**

Arterial pressure displays a circadian rhythm that is entrained to the light-dark cycle, resulting in the...
highest arterial pressures during the night when the activity of rats is the greatest and the lowest arterial pressures during the daytime when rats typically sleep (6, 8). Ingestive behavior follows a similar pattern, i.e., rats primarily consume food during the nighttime (30). Therefore, the present study tests the hypothesis that plasma sodium concentration displays a 24-h rhythm parallel to the MAP rhythm and may contribute to changes in MAP observed throughout the 24-h cycle. In contrast to our predictions, the plasma sodium and arterial pressure rhythms are inversely correlated. The second hypothesis is that the circadian rhythm of plasma sodium is disrupted in SHR fed a basal NaCl diet. The results demonstrate that plasma sodium concentrations are elevated in SHR (compared to WKY) on a basal NaCl diet, but SHR and WKY on the basal NaCl diet display nearly identical plasma sodium rhythms. The present data also test the hypothesis that a high-NaCl diet disrupts the plasma sodium circadian rhythm and may thereby contribute to changes in MAP observed throughout the 24-h cycle. In contrast to our predictions, the plasma sodium and arterial pressure rhythms are inversely correlated. The second hypothesis is that the circadian rhythm of plasma sodium is disrupted in SHR fed a basal NaCl diet. The results demonstrate that plasma sodium concentrations are elevated in SHR (compared to WKY) on a basal NaCl diet, but SHR and WKY on the basal NaCl diet display nearly identical plasma sodium rhythms. The present data also test the hypothesis that a high-NaCl diet disrupts the plasma sodium circadian rhythm and may thereby contribute to NaCl sensitivity in SHR. The results demonstrate that a high-NaCl diet elevates average plasma sodium concentration similarly in both SHR and WKY, but the excess dietary NaCl blunts the normal rhythm of plasma sodium concentration only in SHR.

These results disprove our hypotheses that in rats plasma NaCl concentration increases during nighttime ingestive periods. Plasma NaCl and MAP rhythms were inversely related. Several mechanisms may account for the observed NaCl rhythm. First, sympathetic nervous system activity exhibits a diurnal rhythm similar to MAP and thus it elevates MAP, inducing pressure natriuresis and diuresis during the nighttime and sodium retention during the daytime (16). Second, the plasma sodium rhythm is modified by circulating hormones. The circadian rhythm of plasma sodium is synchronous with the rhythms of circulating hormones, such as aldosterone (9, 17, 21), vasopressin, and oxytocin (31, 34, 51) and the renin-angiotensin system (i.e., angiotensin II (17, 21, 40), angiotensin-converting enzyme (44, 47), and plasma renin activity (17, 19, 21, 26)). All of these rhythms are inverse to the rhythms of renal hemodynamics and excretion (18, 29, 38, 39). Third, feeding and drinking patterns and the kinetics of gastrointestinal sodium absorption likely influence the plasma sodium rhythm. As the rats eat, receptors in the oral/gastric system monitor sodium intake and modify drinking, eating, and renal nerve activity accordingly (7, 10, 27, 46). The net effect of this is to dilute plasma sodium during the intake of food containing high NaCl. In contrast to these relatively rapid effects, the absorption of sodium into the blood is more gradual. Much of the sodium from food is absorbed into the blood at the level of the small and large intestines, and this occurs up to 8 h postprandially. Thus, after late-night eating, the ingested sodium continues to be absorbed during the daytime for several hours (43); because there is little water consumption during this period (12), the protracted absorption of sodium likely contributes to the gradual increase in plasma sodium during the daytime (18, 20, 25, 29, 38, 39, 42, 49).

The present results confirm the hypothesis that plasma sodium concentration is elevated in SHR, compared with WKY fed a basal diet. However, although plasma sodium levels are significantly higher in SHR, it is questionable whether this directly contributes to SHR hypertension. When WKY are placed on a high-NaCl diet, their plasma sodium concentration increases to a level above that of SHR fed the basal NaCl diet, yet WKY display no significant increase in 24-h MAP. This does not totally exclude the possibility that in SHR the elevated plasma sodium concentration contributes to hypertension. SHR lack many of the compensatory mechanisms that WKY display and thus could be more sensitive to an increase in plasma sodium concentration. But, would a 4- to 5-meq increase in plasma sodium be capable of elevating MAP by 20 mmHg? This magnitude of plasma sodium increase is physiologically relevant to the brain, because arginine vasopressin release from the hypothalamus is increased by it (45). Also, an infusion of hypertonic saline that causes an acute 4 meq/l increase in plasma sodium elevates arterial pressure by ~20 mmHg in SHR (35). Furthermore, Li et al. (28) demonstrated that, in control rats, a plasma sodium increase of ~4 meq/l did not alter arterial pressure, but in mRen rats, drinking hypertonic saline increased plasma sodium concentration and arterial pressure. Thus the ability of plasma sodium to affect arterial pressure is related to...
the underlying genetics of the animal studied. Whether this increase is relevant to hypertension in SHR on a basal NaCl diet remains to be elucidated.

The high-NaCl diet elevates plasma sodium concentration in SHR and WKY during the entire day, but the contribution of this increase to NaCl-sensitive hypertension in SHR is less clear. The high-NaCl diet causes a similar rise in plasma sodium concentration in SHR and WKY (~3 meq/l). In contrast, the high-NaCl diet differentially affects the plasma sodium rhythms in the two strains. In SHR the rhythm is significantly blunted (the range is reduced by ~40%), and the large decrease in plasma sodium that normally occurs during the early nighttime (~2000) is absent. In WKY, there was no significant reduction in the plasma sodium rhythm, and the early nighttime decrease in plasma sodium concentration is reduced by <25%. Thus an average daily increase in plasma sodium concentration does not inevitably lead to elevated arterial pressure, but the loss of the normal circadian rhythm for plasma sodium may play a role.

It is unclear what accounts for the blunting of the sodium rhythm in SHR, but possibilities include increased ingestive behavior and/or decreased sodium excretion. The failure of plasma sodium to decrease during the early wake period in SHR may contribute to the development of NaCl-sensitive hypertension by activating osmoreceptors at a time when the brain is increasing sympathetic nervous system activity. There are several mechanisms by which elevated plasma NaCl may contribute to NaCl-sensitive hypertension in SHR. Our previous studies indicate that in SHR, a high-NaCl diet leads to a decrease in norepinephrine release in the anterior hypothalamic nucleus (AHA) and that this leads to NaCl-sensitive hypertension (33). Osmo- and/or sodium receptors in the circumventricular organs of the brain appear to contribute to this decrease in AHA norepinephrine release (36) in SHR on a high-NaCl diet. The present results indicate that plasma sodium concentration is abnormally elevated during the early nighttime, thereby potentially activating a sympatoexcitatory pathway at the same time as other brain mechanisms (e.g., suprachiasmatic nucleus) are increasing sympathetic nervous system activity to meet the demands of the wake period.

Perspectives

The present study is the first demonstration that plasma sodium displays a significant circadian rhythm in rats. Although this rhythm is inverse to the arterial pressure and HR rhythms (6, 8), it is synchronous with the rhythms of sodium excretion (23, 35, 44, 45), renal hemodynamics (38, 39), and circulating antinatriuretic and antidiuretic hormones [e.g., the renin-angiotensin system (17, 19, 21, 40, 44, 47), aldosterone (9, 17, 21), vasopressin, and oxytocin (31, 34, 51)]. Taken together, these results suggest that the circadian arterial pressure and endocrine rhythms act in concert to influence plasma sodium concentration. In SHR compared with WKY on basal NaCl diets, plasma sodium concentration is significantly higher, but the circadian rhythms are similar (1). In contrast, exposure to a high-salt diet increases plasma sodium concentration in both SHR and WKY but disrupts the normal plasma sodium circadian rhythm only in SHR. This dysregulation of plasma sodium rhythm may contribute to NaCl-sensitive hypertension in SHR.

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