The Renin-Angiotensin-Aldosterone System and Circadian Rhythm of Urine Variables in Normotensive and Hypertensive Subjects

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Forty-two middle-age male subjects, aged from 47 to 54 years old, were divided into three groups; 14 normotensives (NT), 16 borderline hypertensives (BHT) and 12 hypertensives (HT). The environmental effects were neutralized as much as possible by keeping their activities synchronized and their plasma and urine variables simultaneously investigated using the same protocol as under normal physiologic conditions.

The circadian rhythms of urinary excretions of Na, K, Cl, creatinine, aldosterone, adrenaline and noradrenaline were analyzed by a multivariate method (cosinor method).

Both systolic and diastolic blood pressure were statistically significantly different among the three groups and body weight was greater in HT than in NT. Other plasma and urine variables examined, however, were not significantly different among the three groups.

A statistically significant circadian rhythm was detected in the urine variables. The rhythm characteristics, such as the mesor, amplitude and acrophase, of the urine variables except for the acrophase of urinary Na excretion were not significantly different among the three groups; the circadian acrophase of urinary Na in HT and BHT appeared significantly earlier than in NT. The pathophysiological and clinical significance of the earlier appearance of the circadian acrophase of urinary Na in hypertensive subjects still remains a unique characteristic requiring further investigation.

IT is well known that the variables relating to blood pressure control, such as the renin-angiotensin-aldosterone system and catecholamines are affected by age, sex and several environmental factors. Weidmann et al. have emphasized that without profound sodium

depletion, age may be more important than sodium excretion as a frame of reference for judging both renin and noradrenaline measurements.¹ We have reported that the plasma renin activity and plasma aldosterone concentration showed a statistically significant inverse correlation with age and were significantly greater in the luteal than in the follicular phase in premenopausal women.²

On the other hand, the endocrine pressor substances have been determined easily and accurately by the development of the radio-immunoassay procedure. However, approximate-

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TABLE I SUBJECTS PROFILE AND LABORATORY DATA (Mean $\pm$ SEM)

	NT	BHT	HT
No. of Subjects	14	16	12
Age (yrs)	51.3 $\pm$ 0.4	50.4 $\pm$ 0.6	50.2 $\pm$ 0.4
Body height (cm)	165.6 $\pm$ 1.1	166.5 $\pm$ 1.3	165.8 $\pm$ 1.5
Body weight (kg)	57.8 $\pm$ 2.0	59.8 $\pm$ 1.5	65.5 $\pm$ 3.1*
Quetelet Index (kg/m ²)	21.10 $\pm$ 0.78	21.76 $\pm$ 0.47	23.72 $\pm$ 0.93*
Systolic B.P. (mmHg)	115 $\pm$ 2.7	130 $\pm$ 2.0**	145 $\pm$ 4.9**
Diastolic B.P. (mmHg)	76 $\pm$ 1.8	84 $\pm$ 0.8**	95 $\pm$ 4.9**
Pulse (/min)	72 $\pm$ 2	77 $\pm$ 2	80 $\pm$ 2*
Serum Na (mEq/l)	142.0 $\pm$ 1.0	141.5 $\pm$ 0.5	141.2 $\pm$ 0.4
Serum K (mEq/l)	5.24 $\pm$ 0.14	5.09 $\pm$ 0.09	4.97 $\pm$ 0.10
Serum Cl (mEq/l)	105.7 $\pm$ 0.9	105.7 $\pm$ 0.4	104.1 $\pm$ 1.6
Serum Creat. (mg/dl)	0.84 $\pm$ 0.03	0.80 $\pm$ 0.03	0.77 $\pm$ 0.03
PRA (ng/ml/h)	2.04 $\pm$ 0.54	2.19 $\pm$ 0.50	1.70 $\pm$ 0.34
PAC (ng/dl)	6.3 $\pm$ 0.8	5.3 $\pm$ 0.5	7.3 $\pm$ 0.8
PAd (pg/ml)	43.9 $\pm$ 5.5	46.9 $\pm$ 9.3	50.0 $\pm$ 10.1
PNA (pg/ml)	208.1 $\pm$ 16.7	216.1 $\pm$ 16.6	208.3 $\pm$ 13.1
ACTH (ng/dl)	6.03 $\pm$ 0.88	5.88 $\pm$ 0.92	4.82 $\pm$ 0.68
Cortisol (μ g/dl)	21.0 $\pm$ 1.5	21.9 $\pm$ 1.6	25.0 $\pm$ 2.3
UNaV (mEq/day)	144.5 $\pm$ 9.1	138.5 $\pm$ 8.2	134.0 $\pm$ 7.2
UKV (mEq/day)	36.8 $\pm$ 2.1	37.1 $\pm$ 1.8	36.3 $\pm$ 1.9
UCIV (mEq/day)	124.8 $\pm$ 8.0	130.6 $\pm$ 8.2	121.8 $\pm$ 4.8
UcrV (mg/day)	1503 $\pm$ 57	1580 $\pm$ 38	1615 $\pm$ 60
AER (μ g/day)	4.9 $\pm$ 0.6	3.9 $\pm$ 0.5	5.7 $\pm$ 0.8
UAdV (μ g/day)	7.0 $\pm$ 1.0	9.6 $\pm$ 1.5	7.5 $\pm$ 0.8
UNAV (μ g/day)	36.3 $\pm$ 2.1	36.4 $\pm$ 2.9	36.0 $\pm$ 3.5

NT = normotensive; BHT = borderline hypertensive; HT = hypertensive subjects

* $p < 0.05$, ** $p < 0.01$ (vs. NT)

ly 10% of between-assay variation is unavoidable.

We investigated the age- and sex-matched normotensive and hypertensive subjects, and neutralized the environmental influences as much as possible by keeping the activities of the subjects synchronized. We also analyzed the individual circadian rhythms of urine variables using a multivariate method (cosinor method)^{3,4} and compared the circadian rhythm characteristics among normotensive, borderline hypertensive and hypertensive subjects.

MATERIALS AND METHODS

The subjects were 42 middle-age men, aged from 47 to 54 years old, who volunteered with informed consent for the protocol. Those having proteinuria, glucosuria, hypertensive retinopathy (KW III or above), renal impairment or diastolic blood pressure of 110 mmHg or more were excluded from the study. They had taken neither antihypertensive drugs nor any other regular medications.

Each study was performed in an isolated controlled environment for 2 days. They were all active during the day and rested at night. A rigorous standardization of their activities, such as their times of rising and retiring and their meal times, was kept during the 24-hour period of the study day.

Blood pressure was measured at 06:00 in the recumbent position and 12:00, 18:00, 20:00 and 22:00 in the sitting position.

Urine collections were made at 4-hour intervals for the ensuing 24 hours. The values for the 24-hour urine excretion were calculated as the sum of the 4-hour urine collections.

The blood samples were drawn into chilled vacutainer tubes containing EDTA for plasma renin activity (PRA), EGTA for plasma adrenaline (PAd) and noradrenaline (PNA) and heparin for plasma aldosterone concentration (PAC), cortisol and ACTH, respectively. They were centrifuged at 4°C, 3,000 rpm for 15 min. The plasma was separated and frozen at -20°C until assayed.

TABLE II CIRCADIAN RHYTHM OF URINE VARIABLES

Variables	Group	Mesor	%Amplitude	Acrophase		p
				Degree	Hour	
UNaV		(mEq/4h)				
	NT	24.0 ± 0.8	21.7 ± 11.6	295	19:38	*
	BHT	23.1 ± 0.7	32.8 ± 10.4	225#	15:00#	*
	HT	22.5 ± 0.8	21.0 ± 12.8	237#	15:46#	*
UKV		(mEq/4h)				
	NT	5.97 ± 0.11	25.1 ± 7.1	212	14:07	*
	BHT	6.31 ± 0.14	32.3 ± 7.6	200	13:18	*
	HT	5.85 ± 0.12	24.1 ± 7.1	197	13:07	*
UCIV		(mEq/4h)				
	NT	20.7 ± 0.7	13.9 ± 11.1	249	16:37	*
	BHT	22.2 ± 0.7	38.8 ± 10.4	203	13:33	*
	HT	22.5 ± 0.8	27.0 ± 13.6	204	13:35	*
AER		(μg/4h)				
	NT	0.81 ± 0.02	48.8 ± 10.1	164	10:54	*
	BHT	0.61 ± 0.02	45.5 ± 9.9	153	10:12	*
	HT	0.93 ± 0.04	43.5 ± 16.0	145	9:41	*

NT = normotensive; BHT = borderline hypertensive; HT = hypertensive subjects

*p < 0.01, rhythm detection by zero amplitude test

#p < 0.01, by Student's t-test (NT vs. BHT or HT)

Serum and urinary Na and K concentrations were determined by flame photometry with lithium as the internal standard. Serum and urinary creatinine concentrations were measured by routine autoanalyzer methods. Serum and urinary Cl were measured by a Chloride Counter.

PRA,⁵ PAC⁶ PAd and PNA,⁷ plasma cortisol,⁸ ACTH⁹ and aldosterone excretion rate (AER)¹⁰ were determined by the radioimmunoassay previously reported. Urinary adrenaline (UAdV) and noradrenaline excretion (UNAV) was measured by high performance liquid chromatography and native fluorescence measurements.¹¹ Each individual variable of a given subject was determined in the same assay run.

The single cosinor procedure^{3,4} was used to assess any circadian variation; a cosine curve with a period selected on the basis of a priori knowledge was fitted to the data using the method of least squares. A F statistics was then used to test the zero amplitude hypothesis for a circadian rhythm. For statistically validated rhythms (p < 0.05), the following parameters were estimated: the mesor (rhythm-adjusted mean), the acrophase (lag of crest time in the best fitting cosine function in relation to a given reference time) and the amplitude (half of the total extent of predictable change). The ampli-

tude in this paper is expressed as a percentage of mesor of the urine variables.

The data were expressed as the group means with standard error of the mean (SEM). The t-test was used to examine any significant change.

RESULTS

1) Subjects' classification

The subjects were divided into 3 groups according to their blood pressure level. They were defined as normotensive (NT) when their blood pressure was always below 140 mmHg systolic and 90 mmHg diastolic, and as hypertensive (HT) when their blood pressure was always 160 mmHg systolic and/or 90 mmHg diastolic or more. The remaining subjects fell into a borderline hypertensive group (BHT). Fourteen subjects fell into NT, 16 BHT and 12 HT according to our criteria.

2) Subjects profile and laboratory data

Their profile and all laboratory data examined are shown in Table I. Age and body height were not different among the three groups, and body weight and Quetelet index were statistically significantly greater in HT than in NT. Both systolic and diastolic blood pressure were also

Fig.1. Circadian rhythm of urinary sodium excretion in normotensive, borderline hypertensive and hypertensive subjects. NT = normotensive; BHT = borderline hypertensive; HT = hypertensive subjects * $p < 0.01$

Fig.2. Circadian rhythm of urinary noradrenaline excretion in normotensive, borderline hypertensive and hypertensive subjects.

significantly different among the three groups as shown in Table I.

Neither the serum concentrations of Na, K, Cl and creatinine nor PRA, PAC, PAd, PNA, ACTH and plasma cortisol were statistically significantly different among the three groups.

Urinary Na (UNaV), K (UKV), Cl (UCIV) and creatinine excretion (UcrV), AER, UAdV and UNAV were not also significantly different among the three groups.

3) Circadian rhythm of urine variables

All urine variables analyzed showed statistically significant circadian rhythm ($p < 0.01$ in all of the variables). The mesor and %-amplitude of

UNaV, UKV, UCIV and AER did not show any significant differences among the three as shown in Table II. Although the circadian acrophase of UCIV appeared about 3 hours earlier in both BHT and HT than that in NT, it did not show any statistically significant difference between NT and BHT or HT.

As shown in Fig. 1 and Table II, the circadian acrophase of UNaV in BHT and HT appeared at 15:00 and 15:46, respectively. These were significantly earlier than that in NT, which appeared at 19:38.

The circadian rhythm characteristics, such as mesor, acrophase and amplitude, of UNaV and UAdV were quite similar among the three groups. The circadian rhythm of UNaV in the three are shown in Fig. 2.

4) Correlation between the circadian acrophase of UNaV and other related variables

No significant correlation was detected between the circadian acrophase of UNaV and systolic or diastolic blood pressure in each of the three groups. However, a weak but significant inverse correlation was detected between the circadian acrophase of UNaV and diastolic ($r = -0.38$, $p < 0.02$) or mean blood pressure ($r = -0.34$, $p < 0.05$) in all of the three groups. There was no significant correlation between the circadian acrophase of UNaV and PRA, PAC, AER or plasma and urinary catecholamines.

DISCUSSION

The renin-angiotensin-aldosterone system and catecholamines are influenced by many factors such as age, sex, salt-intake, position and activity. Therefore, the results investigated without consideration of these factors could lead to a different conclusion. Our results could not detect any significant difference of the blood and urine variables examined among the three groups under normal physiologic conditions. These results suggest that some type of stimulation will be absolutely necessary to clarify the pathogenesis of hypertension as compared with normotensive controls.

On the other hand, the acrophase in the circadian rhythm of UNaV appeared significantly earlier in HT or BHT than in NT under physiologic conditions. We have observed similar results in younger subjects with borderline hypertension¹² and in in-patients with essential hypertension.¹³ In addition, hypertensive

patients on low-salt intake had a similar circadian acrophase of UNaV to that of normal subjects.¹³ In normal subjects, however, the circadian acrophase of UNaV was not affected by the manipulation of sodium intake.¹³ Ten to 12g per day of salt intake might represent sodium-loading for hypertensive subjects and could have caused the early appearance of the circadian acrophase in UNaV; this phenomenon is considered to be a kind of exaggerated natriuresis.¹⁴

A weak but significant correlation was detected between the circadian acrophase of UNaV and diastolic or mean blood pressure. The level of the blood pressure may have been influenced to some extent by the early appearance of the circadian acrophase of UNaV. In patients with primary aldosteronism, however, the circadian acrophase of UNaV appeared at around midnight, 6 hours later as compared with that of normal subjects, and returned to normal after surgical removal of unilateral adrenocortical tumor.¹⁵ These results suggest that the mere presence of a high blood pressure level would not account for the early appearance of the circadian acrophase of UNaV in hypertensive subjects.

We propose that this approach may provide additional information to clarify the mechanism of sodium metabolism in the development of hypertension.

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