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Circadian Rhythm of Circulating Aldosterone in Spontaneously Hypertensive Rats[1] (41161)

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Abstract. Circulating levels of aldosterone were measured by radioimmunoassay at hourly intervals during a 24-hr period to establish the diurnal rhythm of this mineralocorticoid in spontaneously hypertensive rats (SHR). Aldosterone levels exhibited a distinct circadian variation with concentrations reaching a zenith at 2000 hr and a nadir at 1200 hr in male and female SHR. Aldosterone levels in females were consistently greater than males. Nocturnal aldosterone levels were highest in the early morning hours in male and moderately elevated in female SHR. Male SHR have significantly higher blood pressure than female SHR.

It has been established that the pathogenesis of high blood pressure in the spontaneously hypertensive rat (SHR) resembles the pathogenesis of essential hypertension as it occurs in man. Although the pathophysiology of the abnormally high blood pressure in SHR is associated with left ventricular hypertrophy, increased cardiac output, volume expansion, and peripheral vasoconstriction, it is our contention that nutrition and adrenal:gonadal hormones play a conditioning role in the pathogenesis of SHR hypertension (1–5). For example, a high caloric:high cholesterol diet or the opposite extreme of semi-starvation will cause inhibition of the development of the naturally occurring hypertension in SHR (6). We (1–5) and others (7) have found that hypophysectomy, adrenalectomy, and early gonadectomy will also effectively inhibit the development of hypertension in SHR. Although adrenal steroid levels are similar in SHR and in normotensive strains of rat under quiescent conditions, SHR are hypersensitive to relatively innocuous stress and they secrete extra quantities of prolactin, corticosterone, deoxycorticosterone, and aldosterone, when challenged (8). In a previous report, we described the diurnal changes in circulating corticosterone and prolactin in male and female SHR (9). In this report, we describe the circadian rhythm of the potent mineralocorticoid aldosterone under quiescent conditions in male and female SHR at the age when high blood pressure in SHR rises most acutely.

Materials and Methods. Male and female, spontaneously hypertensive rats were raised in our Animal Research Colony on a light–dark cycle of 12 hr illumination and 12 hr darkness. The animal quarters were lighted from 0700 to 1900 hr, room temperature was maintained at 26 ± 1°, and humidity at 45 to 50%. Rat chow and water were provided *ad libitum*. Our strain of SH rats were derived from breeder stock kindly provided by Dr. C. T. Hansen, Animal Genetics Division, NIH. The NIH breeder stock were direct descendants of the Okamoto-Aoki SH rats (10).

At 90–100 days of age when blood pressure is rising steeply, groups of 8–10 SHR were decapitated at each hour of the day. Decapitation was conducted within 30 sec after removing the animal from its cage. Blood was collected in glass centrifuge tubes, centrifuged, and stored at −20°.

Serum aldosterone levels were measured by the method of McKenzie and Clements (11). An intraassay variation of only 2.0% was determined for duplicate samples

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within a single assay. An interassay coefficient of variation was calculated to be less than 15%. Coefficients of variation were calculated by the method of Abplanalp *et al.* (12). The results were analyzed by analysis of variance (13) and by a modification of Duncan's multiple-range test (14).

Results. Serum aldosterone levels in male and female SHR exhibited a distinct rhythm with the nadir occurring at 1200 hr ($P < 0.01$) and the zenith at 2000 hr ($P < 0.05$) (Fig. 1).[3] Aldosterone levels were significantly greater ($P < 0.05$) at 1600, 2400, and 400 hr in female SHR compared to males.

Discussion. These findings demonstrate that there is a definite circadian rhythm in the secretion of the potent mineralocorticoid aldosterone in male and female SHR. Investigation of the diurnal rhythm of aldosterone in animals and in man indicate that the actual secretion of aldosterone, like corticosterone, occurs in episodic bursts. This would account for the relatively wide range of standard error, particularly the nocturnal levels when rats are most active. It must be emphasized that the determination of the circadian secretion of aldosterone in these SHR was done under strict conditions of quiescence. Animals manifesting any evidence of stress due to handling were discarded. Decapitation does not affect circulatory aldosterone levels. Our evidence demonstrates that under quiescent conditions adrenal steroid secretion in SHR is equal to or slightly above that of other strains of normotensive rats (8). As SHR mature, they become extra responsive to stress, i.e., extra production of prolactin, aldosterone, deoxycorticosterone, and corticosterone, concomitant with their progressively increasing levels of blood pressure (8). Our finding of apparently normal circadian rhythm of aldosterone secretion does not negate our contention of pituitary–adrenal participation in the pathogenesis of their spontaneous hypertension. It is the hyperreactivity of SHR and their extra responsiveness to stress which we contend may be the mediating factor in their genetically programmed hypertension.

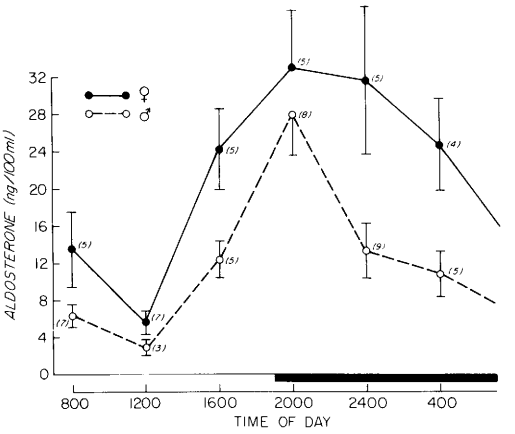

FIG. 1. Serum concentrations of aldosterone in the female (●——●) and male (○– – –○) SH rat during a 24-hr period. Shown are the mean ± SEM; The number of rats per group are in parentheses. The darkened area on the abscissa indicates the period of darkness.

The higher diurnal levels of aldosterone in female SHR is characteristic of most strains of rodents. However, the higher secretory levels of aldosterone in female SHR do not correlate with the fact that during the acute ascent of blood pressure in SHR, the blood pressures of male SHR are considerably higher (10 to 15 mm Hg) than in females. Morris *et al.* (16–20) have shown that although female rats have higher circulating levels of aldosterone, they concomitantly have a higher excretion rate of aldosterone than males. Male rats are more responsive to aldosterone than female rats (16–18). These investigators have also shown that the metabolic sojourn of aldosterone in the rat can be altered greatly by sex steroids or castration (19, 20). Our substrain of SHR is prone to develop premature hypogonadism (1–5). The proclivity for premature hypogonadism in SHR may alter the normal adrenocortical biosynthesis of aldosterone and concomitant high blood pressure. The greater production of aldosterone, coupled with the greater production of corticosterone in female SHR

[3] To establish a nadir or zenith reference, we subjected all of the data to analysis of variance. By using the statistical method, "Treatment of the Means" (C. Y. Kramer, Biometrics **12**, 207, 1956), we were able to determine which means were significantly above or below an "average" mean.

(9), is in keeping with the significantly heavier adrenal glands of female vs male SHR. The latter relationship is also true in normotensive strains of rat.

The diurnal changes in circulating aldosterone parallel the synchronous secretory pattern between blood corticosterone and prolactin in SHR (9). Aldosterone, prolactin, and adrenal steroids affect electrolyte metabolism as well as blood pressure levels. Therefore, the diurnal rhythm of aldosterone, prolactin, and other adrenal steroids in the pathogenesis of hypertension in SHR bears further investigation.

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