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Circadian Changes in Plasma Renin Activity and Plasma Aldosterone Concentration in One-Kidney Hypertensive Rats (41312)

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Abstract. One-kidney hypertension (1KH) was produced in rats by placing a clip on one renal artery and removing the other kidney; control rats were uninephrectomized. On Days 4, 8, and 16 after clipping, groups of 1KH and control rats were sacrificed at 8 AM, 4 PM, and 12 midnight (MN) and blood was collected for measurements of plasma renin activity (PRA) and plasma aldosterone concentration (PAC). Systolic blood pressures for all groups of 1KH rats were elevated above those of the corresponding control rats. PRA was elevated ($P < 0.025$) in the clipped rats on Day 4 at all three times (8 AM, 4 PM, MN); PRA was not elevated ($P > 0.05$) on Days 8 and 16 at any time. Circadian variations in PRA were similar in both 1KH and control group on all 3 days with the peak value observed at 4 PM. PAC was elevated ($P < 0.025$) in the clipped rats on Days 4 and 8 at all times except for MN on Day 4; PAC was not elevated ($P > 0.05$) on Day 16 at any time. A circadian rhythm for PAC was destroyed in clipped rats and appears distorted in the control rats on Day 4; by Day 16, however, both control and clipped rats demonstrated a circadian rhythm for PAC with the peak at 4 PM. It is concluded that normal circadian variations in PRA and PAC are altered only transiently during the pathogenesis of 1KH in rats.

In a previous report from this laboratory (1), the circadian variations for plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were studied in both normal and two-kidney Goldblatt hypertensive rats; the hypertensive rats were observed 4, 5, 7, and 10 weeks after constriction of the renal artery. Both PRA and PAC were elevated in all four hypertensive groups when compared with the normal controls. Moreover, the normal circadian rhythm for PRA was obliterated in the hypertensive groups but for PAC the circadian rhythm persisted (1). Grim and Keitzer (2) have presented evidence suggesting that the normal circadian rhythms for PRA and PAC also are distorted in patients with unilateral renovascular hypertension.

Circadian variations in PRA and PAC have not been reported for the one-kidney Goldblatt hypertensive rat model. The purpose of this study was twofold: (i) to determine any changes in the circadian pattern for PRA and PAC in the one-kidney Goldblatt hypertensive rat; and (ii) to determine when PRA and PAC return to control levels in one-kidney hypertensive rats.

Methods. All rats (Spraque–Dawley males) were housed individually in metabolic cages and kept in temperature-controlled rooms which were maintained on a 12-hr light–dark cycle beginning at 5 AM. Water and powdered Purina rat chow (sodium content: 0.137–0.151 meq/g; potassium content: 0.245–0.268 meq/g) were available *ad libitum*. On the day before sacrificing the rats, systolic blood pressures were measured in conscious rats by the tail artery occlusion method; the recorded value was the average of three or four successive determinations.

Hypertensive studies. Three experimental groups and three control groups with 22–36 male rats in each group were used in this study. The rats (body weight, 130–150 g) were lightly anesthetized with ether and a rigid silver clip with an internal diameter of 0.22 mm was placed on the left renal artery; the right kidney was then removed. Sham control animals underwent identical procedures except that no clip was placed on the renal artery. One experimental group and one control group were sacrificed on Days 4, 8, and 16 after surgery and clipping. Each group was randomly subdivided into three smaller subgroups of 7 to 12 rats for subsequent decapitation and collection of

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blood for hormonal analyses. The rats were decapitated at 8 AM, 4 PM, and 12 midnight (MN). Plasma renin activity (PRA) was determined from blood (2 ml) collected during the first 5 sec after decapitation, while plasma aldosterone concentration (PAC) was measured from a second blood sample (2–3 ml) collected during the next 10–15 sec.

Analytical methods and statistics. Blood samples for determination of PRA and PAC were collected in tubes containing disodium ethylenediaminetetracetate (EDTA) and immediately placed on ice. The samples were centrifuged and the plasma was stored frozen until the time of assay for PRA and PAC.

Both PRA and PAC were determined by radioimmunoassay procedures described previously (3–5). Briefly, plasma aldosterone samples were extracted with methylene chloride and this extract was placed on small celite columns with 40% ethylene glycol–water as the stationary phase. After elution of the other steriods, aldosterone was eluted with ethylacetate:isooctane (60:40). The eluted aldosterone was quantified by radioimmunoassay (3, 4) and PAC is expressed as nanograms (ng) per 100 ml. Plasma was prepared for the determination of PRA in the following manner. Plasma samples were dialyzed against phosphate buffer (pH 5.3) for 18 hr (three changes). After sodium chloride and diisopropylfluorophosphate (DFP) were added, the samples were incubated at 37° for 60 min to generate angiotensin I. Angiotensin I content was quantified by radioimmunoassay and PRA is expressed as nanograms (ng) of angiotensin I per milliliter (ml) per hour (3, 5).

Statistical analysis of the data was determined by analysis of variance, least significant difference (LSD) test, and Student's unpaired *t* test.

Results. Systolic blood pressure averaged 106 ± 1, 107 ± 2, and 102 ± 2 mm Hg in the three separate sham control groups of one-kidney rats (Fig. 1). Placing a 0.22-mm clip on the left renal artery increased blood pressure to 127 ± 2, 144 ± 4, and 147 ± 6 mm Hg ($P < 0.001$ for all three groups) for the 4-, 8-, and 16-day hypertensive groups, respectively (Fig. 1). No significant differences ($P > 0.05$) were observed in the mean values for pressures recorded on rats sacrificed at 8 AM, 4 PM, and MN within any group.

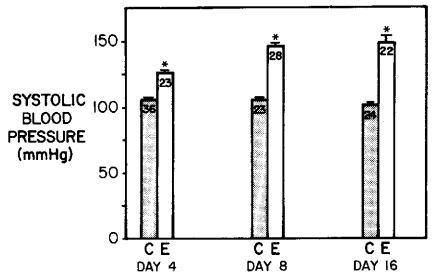

FIG. 1. Systolic blood pressures in control (C) and clipped (E) rats measured one day before sacrifice on Days 4, 8, and 16. The number of rats in each group is shown numerically within the column near the top. Asterisks denote significant differences ($P < 0.001$) between control and clipped groups.

The circadian variations in PRA for all six groups of rats are presented in Fig. 2. As indicated by the asterisks, the 8 AM and 4 PM PRA values were significantly elevated ($P < 0.05$) above the MN PRA values for the control groups on Day 4 and on Day 16 after surgery. There were no statistically significant differences ($P > 0.05$) observed in the circadian variations of PRA for the

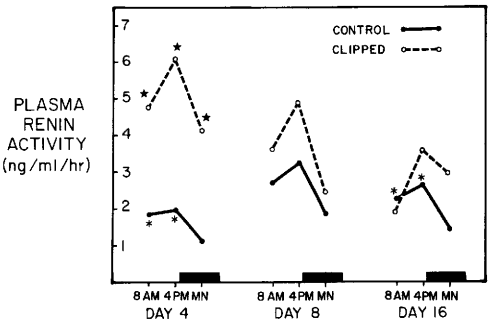

FIG. 2. Circadian variations in one-kidney rats. Plasma renin activity in control (●) and clipped (○) rats sacrificed at 8 AM, 4 PM, and 12 midnight (MN) on Days 4, 8, and 16 following surgery and clipping. Asterisks denote significant elevation ($P < 0.05$) above the MN value within the group; filled stars denote significant differences ($P < 0.025$) between clipped and control groups.

control group on Day 8 or for any of the three clipped hypertensive groups (Fig. 2). Comparisons between clipped hypertensive and control groups revealed that PRA was significantly elevated ($P < 0.025$) in the clipped rats on Day 4 at all three times (Fig. 2). However, there were no significant differences ($P > 0.05$) observed for PRA between clipped and control groups at any time on days 8 and 16 after clipping.

The circadian variations in PAC for all six groups of rats are presented in Fig. 3. As indicated by the asterisks, the 4 PM and MN PAC values were significantly elevated ($P < 0.05$) above the 8 AM PAC values for the control groups on Day 4 and on Day 16 after surgery. Only the MN PAC value was elevated above the 8 AM value ($P < 0.05$) for the control group on Day 8 after surgery (Fig. 3). Similarly, the 4 PM and MN PAC values are significantly elevated ($P < 0.05$) above the 8 AM PAC for the clipped hypertensive groups on Days 8 and 16 after clipping. There were no statistically significant ($P > 0.05$) circadian variations of PAC for the clipped hypertensives on Day 4. Except for the 12 MN PAC values on Day 4 (Fig. 3), the PAC was significantly greater ($P < 0.025$) in the clipped hypertensive rats than in control rats at all times on Days 4 and 8 after clipping. However, there were no significant differences ($P > 0.05$) observed for PAC between clipped and control groups at any time on Day 16 after clipping.

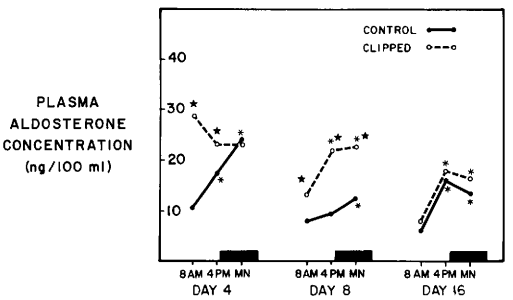

FIG. 3. Circadian variations in one-kidney rats. Plasma aldosterone concentration in control (●) and clipped (○) rats sacrificed at 8 AM, 4 PM, and 12 midnight (MN) on Days 4, 8, and 16 following surgery and clipping. Asterisks denote significant elevation ($P < 0.05$) above the 8 AM value within the group; filled stars denote significant differences ($P < 0.025$) between clipped and control groups.

Discussion. In an earlier study from this laboratory (1), it was demonstrated that both PRA and PAC were higher at 4 PM than at 8 AM or 12 midnight in normal rats. Leenen *et al.* (6) and Gomez-Sanchez *et al.* (7) also observed peak elevations of PRA and PAC during the afternoon hours in normal rats. This earlier study (1) also demonstrated that the normal circadian rhythm for PRA was disturbed in rats with two-kidney, one-clip hypertension of 4 to 10 weeks duration but for PAC the normal circadian pattern persisted.

The present study is the first investigation of the circadian variations in PRA and PAC in rats with one-kidney, one-clip hypertension. Despite surgery and uninephrectomy, a circadian rhythm for PRA was documented in the control rats with the peak value occurring at 4 PM and the low value occurring at 12 midnight; the 8 AM value for PRA also was higher than the 12 midnight value. Similar circadian variations in PRA were observed in the clipped hypertensive rats also, but these were not significant at the 5% level. Thus, the circadian variations in PRA for both the control and the clipped groups remained qualitatively similar to the circadian rhythm for PRA observed previously in the normal rats (1). In contrast, a circadian rhythm for PAC was destroyed in clipped rats and appeared distorted in the control rats on Day 4 after surgery. However, by Day 16 after surgery, both control and clipped rats demonstrated a consistent circadian rhythm for PAC with the peak value occurring at 4 PM; the 12 midnight value of PAC also was higher than the 8 AM value. Thus, it appears that the normal circadian variations in PRA and PAC are altered incompletely and transiently in rats with one-kidney, one-clip hypertension.

In the present study, PRA was significantly elevated in the clipped hypertensive rats on Day 4 but had returned to control levels on Days 8 and 16 after clipping. In contrast, PAC was elevated in the clipped rats on Days 4 and 8 but it returned to control levels on Day 16 after clipping also.

Singer and associates (8) reported that aldosterone secretion was normal in one-kidney, one-clip hypertensive rats that were studied 3 to 18 weeks after the onset of hypertension. McCaa *et al.* (9) reported that both PRA and PAC were slightly suppressed below control levels in benign one-kidney, one-clip hypertensive rats during the chronic phase (>2 weeks of hypertension). Thus, the present data together with these earlier studies suggest that plasma renin and aldosterone are increased only transiently in one-kidney, one-clip hypertensive rats. Interestingly, Freeman *et al.* (10) were unable to block the pathogenesis of one-kidney, one-clip hypertension in rats with continuous blockade of the renin–angiotensin system with captopril. However, the onset of hypertension in these one-kidney, one-clip rats was delayed for 4 to 8 days during blockade with the converting enzyme inhibitor captopril.

In conclusion, the present study demonstrated transient and incomplete circadian rhythm changes for PRA and PAC in rats during the pathogenesis of one-kidney, one-clip Goldblatt hypertension. By Day 16 after clipping, however, chronic hypertension developed and no significant hormonal changes were observed between hypertensive and control rats.

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