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# A Diurnal Rhythm in Plasma Renin Activity in Man *

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Although it has been known for many years that the renin–angiotensin system is responsive to decreases in renal perfusion pressure (1–3), it was only recently discovered that angiotensin is an important regulator of aldosterone secretion (4–6). Before it was known that angiotensin had a role in the regulation of aldosterone secretion, a large number of studies indicated that experimental or pathological conditions that resulted in a reduction of effective blood volume would stimulate aldosterone secretion (7). Studies have already been published indicating that plasma renin activity and aldosterone excretion both increase in response to sodium deprivation (8–10) and assumption of upright posture (11–13).

In the course of studies attempting to correlate plasma renin activity with aldosterone excretion we found that the rises in plasma renin activity and in urinary aldosterone that occur after the assumption of upright posture early in the day are both of limited duration and that they diminish late in the day despite continued maintenance of upright posture (14). The observed afternoon fall in plasma renin activity is not readily explained by existing knowledge of the physiology of renin secretion.

The present study, therefore, focuses attention for the first time on the question of whether there might be a consistent diurnal rhythm in plasma renin activity. In order to characterize diurnal variations in plasma renin activity that might occur independently of dietary and postural influences, we studied normal subjects during continuous recumbency while they received constant diets in equal portions at regular intervals throughout the day and night. To determine whether the rise in plasma renin activity that occurs in response to upright posture is due to "loss" of blood into the lower extremities, we observed the effect of bandaging the lower abdomen, hips, and legs before the subjects assumed upright posture. In order to determine whether the afternoon fall in plasma renin activity of upright subjects was a function of the time of day rather than the duration of upright posture, we compared the effect of rising at noon with the effect of rising at 8 a.m. We also performed studies to assess the importance of sodium and water intake and of aldosterone secretion in bringing about the afternoon decline in plasma renin activity.

## Methods

*Clinical methods.* Normal subjects were given constant 100 mEq, 30 mEq, or 10 mEq sodium diets for at least 5 days before study. During periods of controlled recumbency, subjects remained flat in bed, were fed lying flat, and voided lying flat. When subjects were upright they walked, stood, or sat but were never recumbent. For determinations of plasma renin activity, between 20 and 30 ml of blood was usually drawn, but this amount was reduced to between 10 and 15 ml during studies requiring frequent sampling from subjects on low sodium diets.

*Laboratory methods.* Plasma renin activity was measuered by the method of Boucher and co-workers (15, 16) modified in the following manner. The anticoagulant used was 0.2 ml of 10% ammonium EDTA. The resin was added to the plasma before the pH was adjusted to 5.5. The final product was taken up in 1 ml of 5% bovine albumin solution rather than 1 ml of 20% ethanol, since the latter was itself found to cause a small rise in blood pressure in the rat. The rats were anesthetized with urethan-NF, and autonomic cardiovascular adjustments were blocked with a combination of phenoxybenzamine hydrochloride and pentolinium tartrate. The pressor activity of each unknown sample was compared

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at two dose levels with that of standard angiotensin amide (Hypertensin). Each sample was tested in this fashion in each of two rats. Results were expressed as micrograms angiotensin generated per 100 ml plasma per 3 hours' incubation.

*Reproducibility and recovery.* Replicate determinations using samples from plasma pools gave results with a coefficient of variation of 12%. When 60 ng of standard angiotensin was added to plasma samples immediately before incubation, the mean recovery was 82%, with 95% confidence limits of 74 to 90%. This degree of recovery was possible because of the protection afforded by adsorption onto the resin, since if angiotensin was added at a stage before addition of resin, even if the pH had already been adjusted to 5.5, recovery was markedly reduced. Without use of the resin, such measures as cooling, removal of calcium ions by ammonium EDTA, and pH adjustment to 5.5 proved to be inefficient for angiotensinase inhibition.

*Sensitivity.* Rats of suitable sensitivity for assaying small amounts of angiotensin responded to 0.2 ng of standard angiotensin with at least a 4 mm Hg rise in blood pressure, which was of sufficient magnitude to be consistently distinguishable from spontaneous fluctuations in blood pressure. This meant that the lower limit of sensitivity of this assay method permitted the detection of 25 ng of angiotensin generated per 100 ml of plasma when 0.08 ml of extract (representing 0.8 ml of plasma) was injected into a single rat. Therefore, the sensitivity of the method was quite adequate for the present study.

*Specificity.* Norepinephrine, epinephrine, vasopressin, serotonin, and bradykinin, when added to plasma midway through a 3-hour incubation in concentrations estimated to be 10 to 100 times those normally present in peripheral venous blood, could not be detected in the subsequent bioassay.

Because the question of possible inhibitors or accelerators of renin activity was not investigated, the conventional term "plasma renin activity" rather than "plasma renin" has been employed. Ancillary studies performed with the plasmas of several of our subjects provided little or no evidence to suggest that variations in renin substrate concentrations were important. Thus, in studies of ten subjects representing a wide range of plasma renin activity levels, the incubation of amounts of plasma for 1, 2, and 3 hours revealed no distinct departure from linearity in the rate of generation of angiotensin from hour to hour.

Large variations in volume of injection were avoided to preclude the occurrence of volume-related pressor responses. The intravenous injection of 0.08 ml of 5% albumin into a properly prepared rat never induced more than 1 mm Hg change in blood pressure. This was the maximal volume injected at one time in assaying plasma extracts. Ancillary studies indicated, however, that the injection of 0.25 ml of 5% albumin frequently caused changes in blood pressure of as much as 3 to 8 mm Hg. The use of an acetic acid wash (16) and the careful removal of all traces of ammonium acetate by sublimation proved effective in avoiding depressor responses.

## Results

### *Temporal changes in plasma renin activity of recumbent subjects.*

When recumbent posture and diet were held constant, variations in plasma renin activity were small compared to those accompanying assumption of upright posture (*vide infra*). Nevertheless, in six normal subjects, three of whom were observed over 2 consecutive days, plasma renin activity was higher in the forenoon than in the afternoon (Figure 1). It thus appears that there is a normal diurnal rhythm in plasma renin activity that is unexplained by diurnal changes in diet or posture.

### *Effects of upright posture on plasma renin activity*

*Rising at 8 a.m.* The effect of upright posture on plasma renin activity was studied in 15 subjects on liberal salt diet (100 mEq sodium). When they arose at 8 a.m., they all exhibited sharp increases in plasma renin activity. Peak values were observed at 10 a.m. in nine subjects and at noon in six. For the 15 subjects the mean plasma renin activity value at 8 a.m. was 0.3 $\mu$g per 100 ml, and the mean peak value (10 a.m. or noon) was 1.1 $\mu$g per 100 ml.

Despite the fact that they continued to be upright, all subjects experienced decreases in plasma renin activity in the afternoon, so that by 8 p.m. the values were approximately as low as they had been at 8 a.m. (Figure 2).

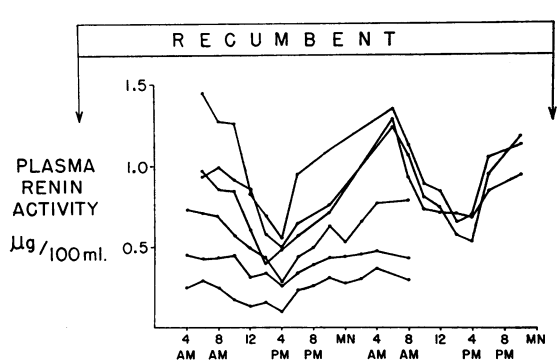


FIG. 1. SERIAL PLASMA RENIN ACTIVITY VALUES OF SIX NORMAL SUBJECTS WHO WERE CONTINUOUSLY RECUMBENT AND WHO RECEIVED IDENTICAL FEEDINGS EVERY 4 HOURS THROUGHOUT THE STUDY. Sodium content of diet, 10 mEq daily.

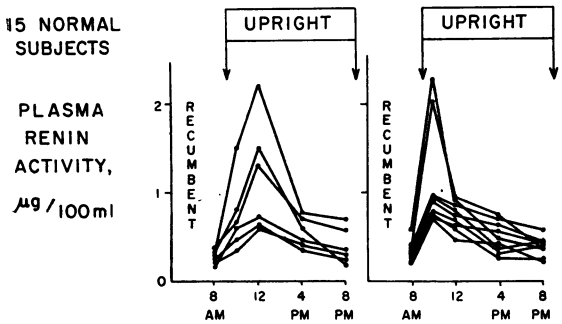


FIG. 2. SERIAL PLASMA RENIN ACTIVITY DETERMINATIONS ON 15 NORMAL SUBJECTS WHO WERE RECUMBENT UNTIL AFTER THE 8 A.M. SPECIMENS WERE DRAWN AND THEN CONTINUOUSLY UPRIGHT UNTIL AFTER 8 P.M. Sodium content of diet, 100 mEq daily.

*Rising at noon.* When subjects on 100 mEq sodium diets rose at noon rather than at 8 a.m., increases in their plasma renin activity were observed but were smaller than those observed when the same subjects rose at 8 a.m. (Figure 3).

*Prevention of the postural rise in plasma renin activity by leg bandaging.* When the lower abdomen, hips, and lower extremities of nine normal subjects (three on 100 mEq sodium intake, three on 30, and three on 10) were firmly bound before they arose at 8 a.m., the usual responses to upright posture were not observed (Figure 4). If the bandages were left in place until noon, their removal was followed by only small increases in plasma renin activity (Figure 4).

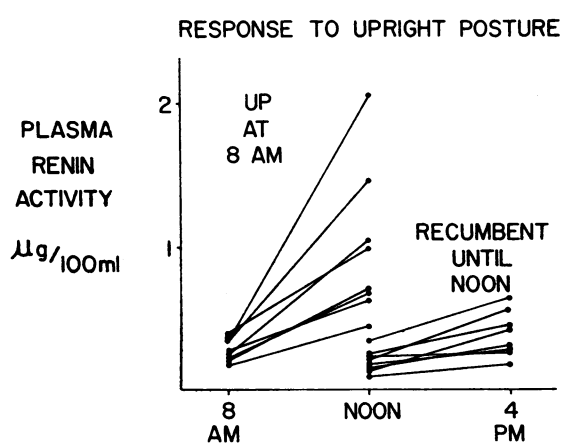


FIG. 3. THE RESPONSE IN PLASMA RENIN ACTIVITY TO UPRIGHT POSTURE IN EIGHT NORMAL SUBJECTS: ON THE LEFT RECUMBENT UNTIL 8 A.M., ON THE RIGHT THE SAME SUBJECTS RECUMBENT UNTIL NOON. Sodium content of diet, 100 mEq daily.

*Nonessentiality of the adrenal cortex in mediating postural changes in plasma renin activity.* In order to test the hypothesis that the afternoon decrease in plasma renin activity was merely a consequence of the fact that aldosterone secretion is high in the forenoon, we studied Addisonian patients so that mineralocorticoid input could be strictly controlled. Three such patients were maintained on constant around-the-clock glucocorticoid and mineralocorticoid replacement therapy, and plasma renin activity was measured before they arose at 8 a.m. and then while they were upright at 10 a.m., noon, and 4 p.m. Despite the fact that mineralocorticoid therapy was given at regular (4-hour) intervals throughout the day, plasma renin activity rose in the morning after the patients had assumed upright posture and fell again in the afternoon (Figure 5).

*Effect of sodium deprivation on plasma renin activity.* Normal subjects who received 10 mEq of sodium per day for several days usually had higher plasma renin activity values than did those receiving liberal sodium diets. In response to assumption of upright posture at 8 a.m. they all exhibited clear-cut increases in plasma renin activity, and in the afternoon they all experienced decreases despite continued maintenance of upright posture. Unlike the subjects on liberal sodium intake, however, those on restricted sodium

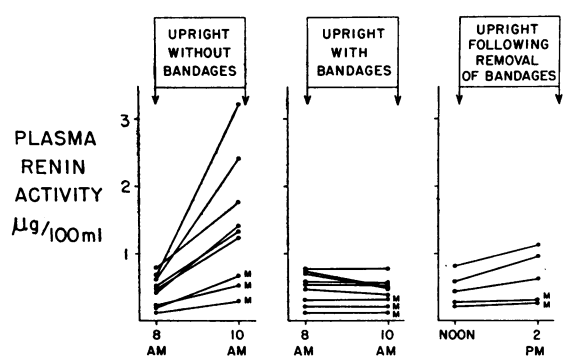


FIG. 4. SERIAL PLASMA RENIN ACTIVITY DETERMINATIONS ON NINE NORMAL SUBJECTS WHO ON ONE DAY AROSE AT 8 A.M. WITHOUT LEG BANDAGES AND ON ANOTHER DAY AROSE AT 8 A.M. AFTER THE LOWER ABDOMEN, HIPS, AND LOWER EXTREMITIES HAD BEEN FIRMLY WRAPPED. Three of the subjects received constant 100 mEq sodium diets (indicated by M), and the others received 30 mEq or 10 mEq constant sodium diets. In five of the subjects, plasma renin activity was measured at 2 p.m. after removal of the bandages at noon (right panel).

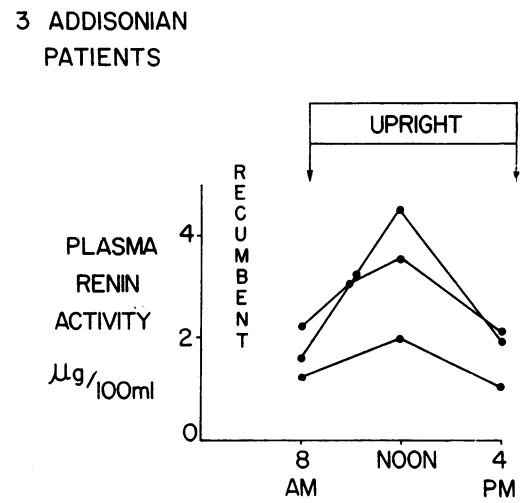


FIG. 5. SERIAL PLASMA RENIN ACTIVITY VALUES OF THREE ADDISONIAN PATIENTS WHO WERE TREATED WITH PREDNISONE, 0.83 MG, AND FLUDROCORTISONE, 0.008 MG, EVERY 4 HOURS. These patients were recumbent until after the 8 a.m. specimens were drawn and upright thereafter. Sodium content of diet, 100 mEq daily.

intake did not show afternoon decreases in plasma renin activity to values as low as those observed at 8 a.m. (upper part of Figure 6). Control of fluid intake so that weight did not change did not alter the pattern of plasma renin activity.

Acute sodium deprivation also partially prevented the afternoon decrease in plasma renin activity of upright subjects. When subjects who had been maintained on 100 mEq sodium diets were totally deprived of dietary sodium for 1 day, their plasma renin activity values rose after assumption of upright posture at 8 a.m. and fell to some extent in the afternoon, but not to values as low as those observed at 8 a.m. (lower part of Figure 6). It should be pointed out that these acutely deprived subjects were in negative sodium balance on the day of the study.

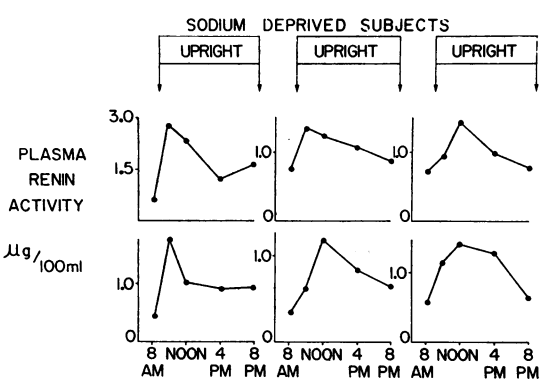


FIG. 6. SERIAL PLASMA RENIN ACTIVITY DETERMINATIONS ON SIX NORMAL SUBJECTS WHO WERE DEPRIVED OF DIETARY SODIUM. The three subjects represented in the upper section each received only 10 mEq of sodium per day for several days before and during the study. The three subjects represented in the lower section each received 100 mEq of sodium daily before the study but none on the day of the study.

## Discussion

The present study has confirmed and extended those of other investigators who have shown that in normal subjects restriction of sodium intake (8) and upright posture (11) increase plasma renin activity. It seems probable that upright posture leads to pooling of blood in the lower extremities, that this loss of effective blood volume results in a fall in renal blood flow, and that this decrease in renal perfusion results in increased production of renin. It has been shown previously that upright posture is associated with a fall in renal blood flow (17), a decrease in urinary sodium (18), and a rise in urinary aldosterone (12–14). By bandaging the lower extremities before the patient assumes upright posture, one can prevent the posturally induced fall in sodium excretion (19) and the posturally induced rise in aldosterone excretion (13). It seems reasonable to assume that it was through the prevention of a fall in renal blood flow that bandaging the extremities prevented a posturally induced rise in plasma renin activity of the subjects in the present study.

The present study describes for the first time a diurnal rhythm in plasma renin activity that is not dependent on diurnal variations in posture or diet. In normal subjects who have no diurnal variation in posture (because they are recumbent at all times) and no diurnal variation in diet (identical feedings every 4 hours) there is, nevertheless, a diurnal rhythm in plasma renin activity with lowest values in the afternoon. When diurnal variations in posture or diet do occur, they can, of course, affect plasma renin activity and, depending upon their timing, can either negate or exaggerate the basic diurnal rhythm in plasma renin activity. It is possible that the diurnal rhythm in plasma renin activity might be secondary to a diurnal rhythm in renal perfusion.

Although renal blood flow was not measured in the present study, it has been reported previously to have a diurnal rhythm with highest values in the afternoon (20). This would offer a plausible explanation for a decrease in renin secretion at this time of day.

Whatever its underlying mechanism might be, the diurnal rhythmicity of plasma renin activity provides an explanation for some otherwise enigmatic findings in the present study. The fact that assumption of upright posture seems to be a much more effective stimulus to plasma renin activity in the forenoon than in the afternoon can be understood if it is recognized that in the afternoon the postural stimulus must work in opposition to the diurnal rhythm. The fact that the midday removal of bandages from the lower extremities of upright individuals was not followed by sharp increases in plasma renin activity can be understood if it is assumed that at this time of day the pooling of blood in the extremities must work in opposition to the diurnal rhythm. A similar explanation can be offered for the fact that subjects who are continuously upright from 8 a.m. until 8 p.m. experience increases in plasma renin activity in the forenoon followed by decreases in the afternoon; the postural stimulus to renin secretion may be constant throughout the day, but in the morning it is augmented and in the afternoon negated by the diurnal factor.

It is well known that upright posture leads to sodium retention in subjects on liberal sodium intake. Retention of sodium would be expected to result in re-expansion of the effective blood volume, thus counteracting the initial stimulatory effect of upright posture on renin secretion. This could be one factor that results in an afternoon decrease in the plasma renin activity values of constantly upright individuals. Such an adjustment of fluid volume is clearly not the only factor that brings about the afternoon decrease in plasma renin activity, however, since a decrease of some degree was observed even in subjects in whom the retention of sodium or fluid had been precluded by restriction of intake.

It has been observed previously that there is a diurnal rhythm in aldosterone excretion of normally active subjects. The dominant factor underlying this rhythm appears to be the stimulus of assuming an upright posture during the day (14). The posturally induced increase in aldosterone output is of limited duration, however, and in the afternoon there is a secondary decrease despite continued maintenance of upright posture. If it is assumed that the renin–angiotensin system is an important mediator of the postural stimulus to aldosterone secretion, then the present study offers an explanation for the fact that aldosterone output by normally active subjects rises in the morning but decreases in the afternoon. Aldosterone production apparently increases in the morning because at that time of day the influence of upright posture works in combination with the diurnal rhythm mechanism to induce renin production. Aldosterone production decreases in the afternoon because the diurnal rhythm mechanism tends to negate the influence of upright posture on renin production during that portion of the day. Although the large increases in plasma renin activity that occur after assumption of upright posture have been shown to be accompanied by increases in aldosterone excretion (12–14), further studies will be necessary to establish whether the more modest variations in plasma renin activity in recumbent subjects described in this study are accompanied by parallel variations in aldosterone excretion.

## Summary

Plasma renin activity of recumbent normal subjects exhibits a diurnal rhythm that is not dependent upon diurnal variations in posture or diet. Highest values are observed between 2 a.m. and 8 a.m. and lowest values between noon and 6 p.m. A change from recumbency to upright posture leads to a greater increase in plasma renin activity in the forenoon than it does in the afternoon. The posturally induced increase in plasma renin activity can be prevented by bandaging the lower abdomen, hips, and lower extremities. When a normal subject rises at 8 a.m., his plasma renin activity increases to peak values at 10 a.m. or noon and then falls despite continuation of upright posture. The afternoon fall in plasma renin activity is not dependent upon changes in adrenocortical function, nor is it entirely dependent upon retention of salt and water during the forenoon. In subjects who are upright during the day, the diurnal rhythm mechanism appears to work in combination with postural factors to elevate

plasma renin activity in the forenoon and to work in opposition to postural factors to depress plasma renin activity in the afternoon.

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