JCI The Journal of Clinical Investigation

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J Clin Invest. 1973;52(9):2272-2277. https://doi.org/10.1172/JCI107414.

Research Article

Plasma aldosterone, cortisol, and renin activity were measured in nine recumbent patients with hyperaldosteronism, including seven with adenomas, one with idiopathic hyperplasia, and one with glucocorticoid suppressible hyperplasia. All had peak values of plasma aldosterone concentration from 3 a.m. to noon and lowest values at 6 p.m. or midnight. This rhythm was similar to the circadian pattern of plasma cortisol in the same patients. When these data were normalized to eliminate the wide variation in ranges of plasma aldosterone and cortisol between individuals, there was an excellent correlation ($r = + 0.87$, $P < 0.005$) between the two hormones. In contrast, plasma aldosterone concentrations did not correlate with plasma renin activity before or after normalization of data.

Short term suppression of ACTH by administration of dexamethasone eliminated the circadian variation of plasma aldosterone in both patients with hyperplasia and in four of five patients with adenomas, while it markedly altered the rhythm in the fifth. Similar doses of dexamethasone were administered to four normal subjects and did not flatten the circadian rhythm of plasma aldosterone.

These data suggest that patients with primary aldosteronism have a circadian rhythm of plasma aldosterone mediated by changes in ACTH.

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ABSTRACT Plasma aldosterone, cortisol, and renin activity were measured in nine recumbent patients with hyperaldosteronism, including seven with adenomas, one with idiopathic hyperplasia, and one with glucocorticoid suppressible hyperplasia. All had peak values of plasma aldosterone concentration from 3 a.m. to noon and lowest values at 6 p.m. or midnight. This rhythm was similar to the circadian pattern of plasma cortisol in the same patients. When these data were normalized to eliminate the wide variation in ranges of plasma aldosterone and cortisol between individuals, there was an excellent correlation ($r = +0.87$, $P < 0.005$) between the two hormones. In contrast, plasma aldosterone concentrations did not correlate with plasma renin activity before or after normalization of data.

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This work was presented in part at the Western Section Meeting of the American Federation of Clinical Research, Carmel, Calif., 1971.

Received for publication 14 December 1972 and in revised form 19 April 1973.

INTRODUCTION

Normal recumbent subjects have highest plasma aldosterone concentrations in the morning and lowest values in the evening (1, 2). Similar circadian rhythms have also been described for ACTH (3) and plasma renin activity (4). The plasma aldosterone rhythm therefore might be causally related to one or both of these stimuli. Two groups (1, 5) have studied these relationships in normal subjects on regular sodium diets and have concluded that the circadian rhythm of plasma aldosterone is primarily mediated by plasma renin activity.

Preliminary data suggested that patients with primary aldosteronism due to adrenal adenomas or adrenal hyperplasia (2) also have a circadian rhythm of plasma aldosterone concentration. The renin-angiotensin system might not be expected to control plasma aldosterone concentration in these patients since plasma renin activity is low and aldosterone production is relatively resistant to alterations in dietary sodium intake (6), posture (7), and infused angiotensin II (8). The possibility that normal concentrations of ACTH might alter aldosterone production in some of these patients has been suggested by a variable decrease in aldosterone production and urinary excretion after suppression of ACTH with glucocorticoids (9) and a marked rise after infusion of ACTH (6, 10, 11).

The present study was designed to examine the relative importance of ACTH and of the renin-angiotensin system in controlling the circadian rhythm of plasma aldosterone in patients with primary aldosteronism.

METHODS

Plasma aldosterone concentration (12) and plasma renin activity (13) were measured by radioimmunoassay. Cortisol was determined by partitioning plasma between benzene, cyclohexane, and water and assaying the more polar fraction with a competitive protein binding technique (14). Over 95% of corticosterone is eliminated by this procedure. Tritiated cortisol was added to plasma to correct for losses in extraction. The intra-assay variation between replicates for each assay was 15% or less. All samples from a patient were run concurrently.

Correlation coefficients (15) between plasma hormone concentrations were computed using both original and normalized values. Normalization was performed by assigning 0% to the lowest and 100% to the highest value of each circadian rhythm and interpolating intermediate values. This was carried out in order to correlate data from several patients without distortions attributable to wide variations in the range of hormone concentrations among individuals.

Clinical studies. Seven patients with primary aldosteronism due to an adrenal adenoma were studied. Each had hypertension, hypokalemia, normal plasma cortisol concentrations and suppressed plasma renin activity. Their plasma aldosterone failed to suppress normally after recumbency and infusion of normal saline (16). The presence of an adenoma was established by surgery or adrenal venography in all but one. This patient had very high plasma aldosterone, sodium, and bicarbonate concentrations, markedly suppressed plasma renin activity, and a very low plasma potassium concentration as is characteristic of patients with an adrenal adenoma rather than hyperplasia (17). Attempts at adrenal venography were unsuccessful, and he is not an operative candidate because of severe coronary artery disease. Two patients with bilateral adrenal hyperplasia were also studied. One patient failed to correct his hypokalemia or hyperaldosteronism by glucocorticoid therapy and had the diagnosis of idiopathic hyperplasia confirmed at surgery. The second patient had glucocorticoid suppressible aldosteronism (18) with hyperplasia demonstrated by adrenal venography. Four healthy normotensive males were used as control subjects.

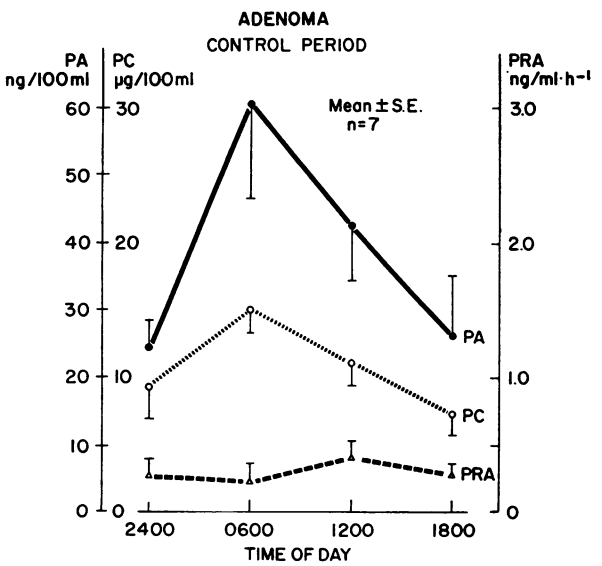

FIGURE 1 Mean plasma aldosterone (PA), cortisol (PC), and renin activity (PRA), during the control period are plotted for seven recumbent patients with primary aldosteronism and adenoma on a regular sodium diet.

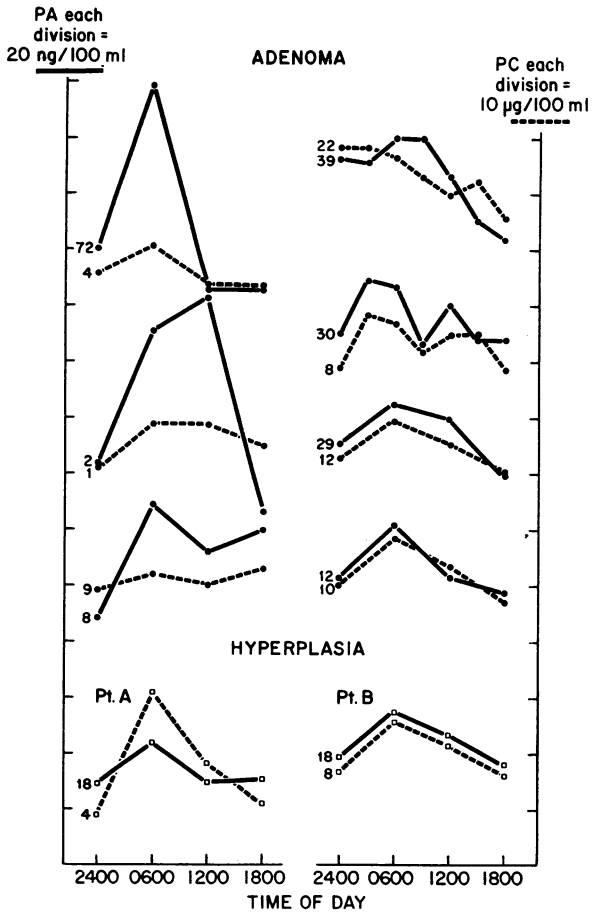

FIGURE 2 The circadian rhythm of plasma aldosterone (PA) and plasma cortisol (PC) have been superimposed for seven patients with primary aldosteronism and adenoma and two patients with hyperplasia. The 2400 hour absolute concentration of each hormone is included. Changes thereafter can be determined from the respective scales. Patient A has idiopathic and patient B has glucocorticoid suppressible hyperplasia.

The studies were performed in the hospital. All patients ate a standard hospital diet and were encouraged to use a similar amount of sodium each day. When potassium supplements were necessary, the amount used was constant throughout the study. Experiments were performed in such a way as to simulate a normal sleep-posture relationship. Patients went to bed at 10 p.m. with an indwelling needle in an arm vein. Speciments were obtained at midnight, 6 a.m., noon, and 6 p.m. The subjects were permitted to sit for 5–10 min immediately following sampling in order to eat and use a bedside commode at 6 a.m. and noon. This routine was felt to provide less stress to the subject than attempting to eat or attend to bodily functions in a recumbent posture and provided almost 6 h of recumbency before the next phlebotomy. This is more than adequate time to

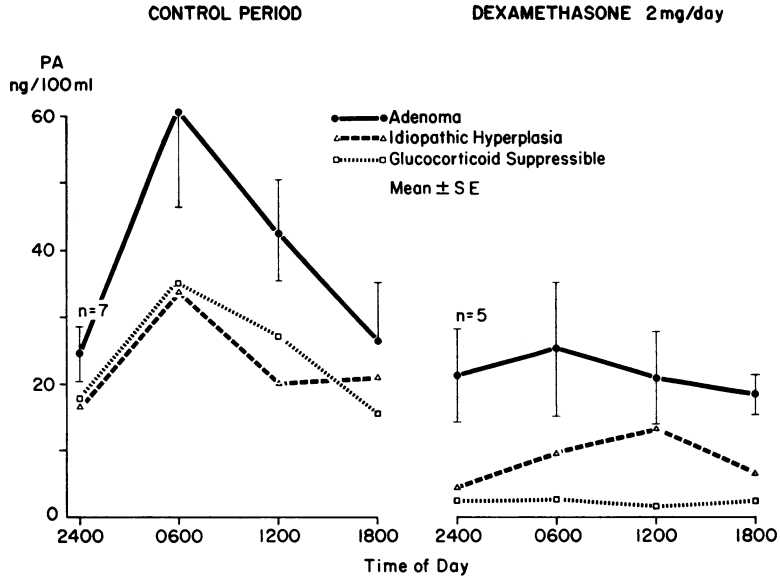

FIGURE 3 Mean plasma aldosterone (PA) at intervals throughout the day are plotted for patients with primary aldosteronism and adenomas during the control period and during dexamethasone therapy. Individual values for the patient with idiopathic hyperplasia and the patient with glucocorticoid suppressible hyperplasia are included for comparison.

establish basal conditions since Katz, Romfh, and Smith (19) found that plasma renin activity and plasma aldosterone returned to basal levels within 1 h of such activity. Blood volume losses during each sampling were only 15 ml. This small volume loss is not sufficient to stimulate hormonogenesis (20).

For studies of circadian variation during acute suppression of ACTH, 0.5 mg of dexamethasone was given by mouth every six h beginning 6 h before the first blood sample was obtained. Patients were recumbent as in the control period. These studies generally were performed the day following the control study. The patients were ambulatory between 6 and 10 p.m. prior to the second study.

RESULTS

The patients with aldosterone-producing adenomas had a distinct circadian rhythm of plasma aldosterone concentration with the peak mean value at 6 a.m. and lowest values at 6 p.m. or midnight (Fig. 1). The peak mean plasma renin activity, however, occurred at noontime. There was no correlation between plasma renin activity and plasma aldosterone concentrations before or after normalization of the data ($r = +0.05$, $P > 0.10$). Most patients with adenomas had circadian rhythms of plasma aldosterone concentration similar to the two patients with hyperplasia (Fig. 2). The rhythms of plasma aldosterone and cortisol for each patient have been superimposed in Fig. 2 and indicate that the changes in hormones are qualitatively similar. When a linear regression coefficient was derived using pooled data from all of the patients, no statistical significance was achieved in spite of the similarity of the two rhythms. Upon inspection of the data, it become apparent that this was primarily due to the marked variation in the range of plasma aldosterone and cortisol concentration between individual patients. When the disparity in the ranges was eliminated by normalizing the data of individual patients before they were pooled, the correlation between plasma aldosterone and cortisol concentrations was highly significant ($r = +0.87$, $P < 0.005$).

Five patients with adenomas, the patient with idiopathic hyperplasia and the patient with glucocorticoid suppressible hyperplasia were also studied while taking dexamethasone (Fig. 3). There was no change in dietary intake between the control and dexamethasone periods. The mean 6 a.m. plasma potassium concentration was 2.8 meq ±0.4 SEM in the control period and 3.0 meq ±0.2 SEM during the dexamethasone period. The patients with adenomas generally had higher concentrations of plasma aldosterone during the control period than did the patients with hyperplasia. As expected, plasma aldosterone concentrations were suppressed to very low values in the patient with glucocorticoid suppressible hyperplasia. The patient with idiopathic hyperplasia had moderate suppression and marked flattening of his circadian rhythm of plasma aldosterone. The five patients with adenomas also had moderate suppression of their mean plasma aldosterone concentration below control values during the 24 h period. The circadian rhythm was flattened in four of these patients and markedly altered in the fifth. While the circadian rhythm of

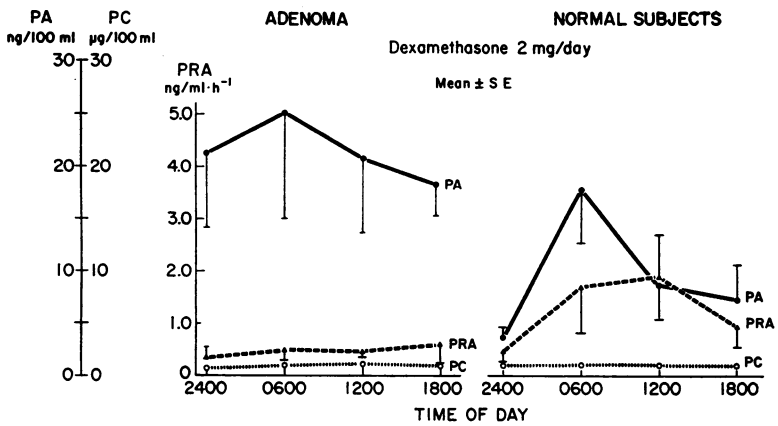

FIGURE 4 Mean plasma aldosterone (PA), plasma cortisol (PC), and plasma renin activity (PRA), are plotted for five recumbent patients with hyperaldosteronism and adenoma during dexamethasone 2 mg/day therapy. Data are similarly plotted for four recumbent normal subjects studied under identical conditions.

plasma cortisol was abolished in these patients with primary aldosteronism after dexamethasone suppression of ACTH (Fig. 4), there was still a poor correlation of plasma aldosterone concentration with plasma renin activity before and after normalization of the data ($r = -0.17$, $P > 0.10$). When four normal subjects were similarly studied, the circadian rhythm of plasma aldosterone was preserved, and it was significantly correlated with changes in plasma renin acitvity ($r = +0.68$, $P < 0.005$).

DISCUSSION

Michelakis and Horton (1) and Williams, Cain, Dluhy, and Underwood (5) have observed a circadian rhythm of plasma aldosterone in normal subjects on a regular diet. The changes in mean plasma aldosterone concentration were closely correlated with changes in mean plasma renin activity, both being highest in the early morning and lowest at 6 p.m. or midnight. Although discrepancies between the two hormones in normal subjects on a low sodium diet were noted in the study by Michelakis and Horton, the conclusion has been reached in other studies by Williams and Dluhy (21) that changes in plasma renin activity are the main determinant of the circadian rhythm of plasma aldosterone in recumbent normal subjects.

In patients with primary aldosteronism, the renin-angiotensin system would not be expected to be an important factor controlling aldosterone production since plasma renin activity is low and infusion of angiotensin II has been reported to be ineffective in increasing aldosterone production in untreated patients (8). The present study confirms the lack of importance of plasma renin activity in the control of plasma aldosterone concentrations in these patients while recumbent since no correlation was noted between the two hormones. Since plasma renin activity does not appear to be a controlling factor under these conditions, significant alterations in the circadian rhythm of plasma aldosterone in patients with primary aldosteronism might be expected. However, we have found a normal appearing circadian rhythm of this hormone in every patient with primary aldosteronism that we have studied although plasma aldosterone concentrations were higher than normal relative to sodium intake and plasma potassium concentration.

Aldosterone excretion and secretion rates in patients with primary aldosteronism due to an adrenal adenoma have been reported as variably diminished or unaffected by administration of glucocorticoids (6, 9, 10, 22). The inconstant effects of ACTH suppression and transient increase of aldosterone secretion (10, 11) or excretion (11) after ACTH stimulation has led some to conclude that ACTH is not a major stimulus to aldosterone production in patients with adenomas. Since secretory rates or urinary excretion represent an integrated value of the daily hormone production, major alterations may occur in the rhythm of aldosterone production and plasma concentration without being detected by these tests.

Our patients with aldosteronism due to adenoma and the patient with idiopathic hyperplasia had a highly significant positive correlation between the concentrations of plasma aldosterone and cortisol throughout the day. This was similar to the expected close relationship of the two hormones found in the patient with glucocorticoid suppressible hyperplasia. This latter patient may represent a form of adrenal enzymatic deficiency that is apparently controlled by ACTH since glucocorticoid administration abolished aldosterone hypersecretion. This

syndrome provides an important model for evaluating the importance of ACTH in patients with hyperaldosteronism.

After dexamethasone was administered to five patients with adenomas and one with idiopathic hyperplasia to suppress endogenous ACTH, the circadian rhythm of plasma aldosterone was flattened in five and markedly altered in the sixth. This flattening of the circadian variation was similar to that seen in the patient with glucocorticoid suppressible hyperplasia. The response of these patients to dexamethasone therapy was in marked contrast to that of the normal subjects. In the latter group, a normal circadian rhythm of plasma aldosterone concentration was observed that closely correlated with changes in plasma renin activity in the absence of ACTH. It is possible that variations in plasma aldosterone have been missed by sampling at 6-h intervals and that this accounts for the marked flattening of plasma aldosterone after dexamethasone therapy. It is unlikely that this would have occurred in all seven patients with hyperaldosteronism and not in any of the four similarly examined normal subjects. The observed changes could not be ascribed to alterations in potassium intake since the diet and potassium supplements, if required, were not changed between the two periods. The mean 6 a.m. plasma potassium concentrations were not significantly different during dexamethasone therapy than during the control period. While we did not measure plasma potassium throughout the day in our patients, Williams et al. (5) did not find a significant circadian rhythm of potassium in their normal subjects. The decrease in the mean plasma aldosterone concentration after 1 day of dexamethasone administration in the patients with adenomas and in the one patient with idiopathic hyperplasia was less than that seen in the patient with glucocorticoid suppressible hyperplasia. At present, we do not know why there are such marked differences in the degree of suppression during dexamethasone therapy.

These observations do not lead us to conclude that primary aldosteronism is due to increased ACTH production. Several studies have failed to develop evidence for an increased production of ACTH in primary aldosteronism (23, 24). Presumably, the abnormal aldosterone producing adrenal tissue has little inherent rhythm of aldosterone production but in the absence of significant levels of angiotensin II does respond to normal or induced fluctuations of endogenous ACTH and of potassium in the diet (10) and in plasma (21).

In summary, we have found that patients with primary aldosteronism have a circadian rhythm of plasma aldosterone concentration that appears similar to the rhythm of plasma cortisol but not to that of plasma renin activity. This observation suggests that ACTH might control the circadian rhythm of plasma aldosterone in these patients. This possibility is strengthened by the observation that the circadian rhythm in the patients with primary aldosteronism, unlike normal subjects, is flattened or markedly altered shortly after administration of dexamethasone to suppress ACTH.

Addendum. Since this manuscript was accepted for publication, the predicted diagnosis of adrenal adenoma in our seventh patient was confirmed by adrenal venography and surgery at Portsmouth Naval Hospital, and by gross and microscopic evaluation of the specimen in our pathology laboratory. The assistance of Dr. Jessie Jones is gratefully acknowledged.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Research Grant 1 RO1 HL 1486301, American Heart Association, Texas Affiliate, U. S. Public Health Service grants HL 14159, HL 06308, USPHS RR-00750, General Research Support, University of Arizona College of Medicine, the Arizona Heart Association, and Tripler Army Hospital Research Funds.

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