Activation of Aldosterone Secretion in Primary Aldosteronism

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ABSTRACT Angiotensin infusion evokes marked increases in aldosterone secretion in primary aldosteronism and little change in secondary aldosteronism. The low plasma renin activity of primary aldosteronism and the elevated plasma renin activity of secondary aldosteronism are thought to account for this differential response. The effect of angiotensin on aldosterone and 18-hydroxycorticosterone secretion was studied during adrenal vein catheterization in seven patients with primary aldosteronism (whose plasma renin activity had been elevated following spironolactone therapy), one hypertensive patient with normal plasma renin activity and normal aldosterone secretion, two patients with secondary aldosteronism who had elevated plasma renin activity, and one anephric patient whose plasma renin activity was 0. Adrenal venous aldosterone and 18-hydroxycorticosterone were measured before and after a ten min sub-pressor angiotensin infusion.

The cells of the aldosterone-producing adenoma (APA) respond to small increases in plasma angiotensin with large increases in secretion of aldosterone and 18-hydroxycorticosterone. The dose of angiotensin capable of evoking this response from the aldosterone-producing adenoma produces little or no change in the secretion of the steroids from nontumorous glands. The augmentation of aldosterone secretion, induced by angiotensin, in primary aldosteronism is due solely to increased secretion by the adenoma and not by the contralateral zona glomerulosa. The increased sensitivity of the aldosterone-producing adenoma is characteristic of the tumor. This response is independent of fluctuations in endogenous plasma renin activity. This sensitivity is not blunted by high plasma renin activity, nor is it a function of tumor mass for the effect is observed in aldosterone-producing adenomas regardless of size. ACTH injection after angiotensin infusion resulted in a marked increase in aldosterone concentration in the effluent from the nontumorous adrenal, but was not capable of producing further increases in aldosterone concentration in the effluent from the APA. In view of this exquisite sensitivity to infused angiotensin, it may be that the small variations in endogenous plasma renin activity that have been observed in primary aldosteronism may be capable of evoking large changes in aldosterone secretion in patients with aldosterone-producing adenomas.

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INTRODUCTION

Although both ACTH (1) and angiotensin (2) can stimulate aldosterone secretion, the renin-angiotensin system appears to be the major factor controlling activation of aldosterone secretion in normal man (3, 4). In general, those factors that increase or decrease plasma renin activity result in comparable increases or de-

creases in aldosterone secretion (5, 6). Only in primary aldosteronism does there appear to be a disparity between aldosterone secretion and plasma renin activity, for in this disease, aldosterone secretion remains elevated whereas plasma renin activity (PRA) remains depressed (7). Plasma renin activity does exhibit postural and day-to-day variations (8) in primary aldosteronism but these changes are thought to be of such a small order of magnitude as to be of no significance in the control of aldosterone secretion. In addition, in patients with primary aldosteronism, aldosterone secretion varies from day to day, albeit at a constantly higher than normal level (9).

The nature of the factors that control aldosterone secretion in primary aldosteronism have never been fully delineated. The purpose of this report is to explore the possible role of angiotensin and ACTH in the control of aldosterone secretion in patients with aldosterone-producing adenomas.

METHODS

The study was divided into three parts.

(1) *Effect of 6-hr angiotensin infusion on the 24-hr aldosterone secretory rate in primary and secondary aldosteronism.* Eight patients subsequently proven to have aldosterone-producing adenomas (APA) and eight patients with secondary aldosteronism, due to accelerated hypertension, were studied to determine the effect of angiotensin infusion on the aldosterone secretory rate.

The eight patients with primary aldosteronism ranged in age from 24–51. All had presented with hypertension (average blood pressure 190/112; range 160/100–220/124) and hypokalemia (mean serum potassium 3.1 mEq/liter; range 2.5–3.4 mEq/liter). The patients with secondary aldosteronism ranged in age from 32–54. These patients were likewise originally evaluated because of hypertension (average blood pressure 196/116; range 170/104–240/140) and hypokalemia (mean serum potassium 3.3 mEq/liter; range 1.9–3.7 mEq/liter). Keith-Wegener grade II–III hypertensive retinopathy was present in all of the patients with secondary aldosteronism, whereas seven of the eight patients with primary aldosteronism had grade I retinopathy. The remaining patient had grade III retinopathy. The patients with secondary aldosteronism had proteinuria and some evidence of impaired renal function, but in all serum creatinine was below 2.0 mg/100 ml. The patients with primary aldosteronism had repeatedly normal urinalyses, normal blood urea nitrogens, normal serum creatinines and creatinine clearance, and normal intravenous pyelograms. Six of the eight patients with primary aldosteronism and all of the patients with secondary aldosteronism had EKG evidence of left ventricular hypertrophy. The only other EKG abnormality that was characteristics of both groups were prominent "U" waves thought to be related to hypokalemia. None of the patients had any cardiorespiratory symptoms at the time of study. None of the patients had taken any medication for at least 4 wk before the study.

After a minimum of 2 days on 120 mEq sodium and 40 mEq potassium diet, control 24-hr aldosterone secretory rate was done. On the following day, angiotensin was infused intravenously in a concentration of 250 mμg/ml at a rate sufficient to elevate the diastolic blood pressure 10 mm Hg for the entire 6 hr of the infusion (8:00 a.m.–2 p.m.). Tritiated aldosterone was injected at 8 a.m. and a 24 hr aldosterone secretory rate (ASR) was done as on the previous day.

(2) *Effect of angiotensin on the secretion of aldosterone from aldosterone-producing adenomas and nontumorous adrenals in vivo.* 11 patients were studied during the course of adrenal vein catheterization. Seven patients had aldosterone-producing adenomas, two patients had secondary aldosteronism due to accelerated hypertension, one patient had essential hypertension but normal plasma renin activity and aldosterone secretory rate, and one patient was anephric and undergoing chronic hemodialysis. Six of the seven patients with primary aldosteronism were included in part one of the study. The seventh patient presented with hypertension (blood pressure 240/140) and hypokalemia (serum potassium 2.5 mEq/liter), but differed from the other patients with primary aldosteronism by virtue of the fact that he had proteinuria, an elevated BUN of 39 mg/100 ml, and an elevated creatinine of 1.9 mg/100 ml. All of the patients with primary aldosteronism had been pretreated for at least 5 wk with spironolactone in a dose of 400 mg/day. The other patients were receiving no medication. The anephric patient had been dialyzed 18 hr before adrenal vein catheterization.

The technique of adrenal vein catheterization has been previously described (10). All studies were carried out between 8:00 a.m. and noon, with the patients recumbent. After collection of 25 ml of blood from each adrenal vein, angiotensin was infused through a peripheral vein at a rate sufficient to maintain the diastolic blood pressure 10 mm Hg above base line. The dose required to achieve this effect in primary aldosteronism averaged 200 mμg/min and exceeded 500 mμg/min in the remaining patients. After a 10 min infusion of angiotensin, 25 ml of blood was again drawn from each adrenal vein. Adrenal venous samples were then analyzed for their content of aldosterone and 18-hydroxycorticosterone.[1]

(3) *Effect of intravenous ACTH on the secretion of aldosterone from aldosterone-producing adenomas and nontumorous adrenals in vivo.* One patient with an aldosterone-producing adenoma was studied to determine the acute effect of ACTH on aldosterone secretion. During the control and angiotensin infusion period, blood was collected for adrenal venous aldosterone as noted above. Immediately after the angiotensin infusion, 0.25 mg of β1-24 ACTH [2] was injected over 30 sec into a peripheral vein. 5 min after this injection, adrenal venous samples were collected and analyzed for aldosterone.

There were no appreciable differences in adrenal blood flow during the collections under the conditions described.

Plasma renin activity was measured in the upright position at noon by the method of Gunnels, Grim, Rubinson, and Wildermann (12).[3] Plasma renin activity at this time in the erect position in normal subjects is 272 ±25 ng/100 ml. Aldosterone secretory rate was measured by the method of Melby, Dale, and Wilson (11) which involves calculation of the secretion rate from the specific activity of urinary tetrahydroaldosterone [4] in the 24 hr collection after injection of

[1] Trivial names used are as follows: aldosterone = 11β, 21-dihydroxy-18-oxo-pregn-4-ene-3, 20 dione; 18-hydroxycorticosterone = 11β, 18,21-trihydroxypregn-4-ene-3,20-dione.

[2] Kindly supplied as Cortrosyn by Dr. Henry Strade, Organon, Inc., West Orange, N. J.

[3] Assays for plasma renin activity were carried out at the New England Nuclear Biomedical Assay Laboratories.

[4] Trivial name used is as follows: tetrahydroaldosterone = 18-oxo-3α, 11β, 21-trihydroxy-5β-pregnane-20-one.

5 μc of 1,2-H³-aldosterone. An extract of $\frac{1}{10}$ of this volume was treated with β-glucuronidase and purified in three thin-layer chromatography systems. Silica gel was used as adsorbent in all three systems, except that in the second system a 2% sodium borate solution was used instead of water for preparation. The plates were developed by single dimensional ascending chromatography in two stages for the first and third chromatograms and one for the second. The thin-layer solvent systems employed were as follows: (*1*) ethyl acetate–acetone–water (80:20:5, v/v); (*2*) acetone–benzene–water (90:10:8, v/v); (*3*) chloroform–methanol–water (90:10:1, v/v). Dried eluates from the last chromatogram were dissolved in 2 ml of absolute methanol. Aliquots were removed for ³H assay in a Packard Tri-Carb liquid scintillation counter. Quantitation was accomplished by cupric acetate oxidation of aldosterone to its corresponding glyoxal and reaction with phenylhydrazine–ethanol–sulfuric acid to form the Porter-Silber chromogen. The secretion rate of aldosterone (μg/24 hr) was calculated by dividing the specific activity of tetrahydroaldosterone (cpm/μg) into the injected tracer dose (cpm). The specific activities of tetrahydroaldosterone and the pH-1-conjugate of aldosterone agree within 1%. More than 80% of the label excreted as tetrahydroaldosterone is recovered in 24 hr. The reproducibility of the method is excellent. Mean tetrahydroaldosterone in 8 replicate determinations on pooled urine was 7.20 μg/150 ml; SD = ±0.26 μg/150 ml, and SE of mean = ±0.06 μg/150 ml. The mean ASR in 32 healthy subjects by this method is 100.8 μg/24 hr with a range of 43–160 μg/24 hr on a dietary intake of 120 mEq sodium and 40 mEq potassium per day. Plasma cortisol was determined by the method of Silber and Porter (13).

Isolation and quantitation of aldosterone and 18-hydroxycorticosterone

Adrenal venous aldosterone and 18-hydroxycorticosterone was measured by a technique requiring development of soda fluorescence.

After separation from red cells and addition of a tracer of 1,2-³H-aldosterone to 10 ml or more of plasma for recoveries, the plasma was extracted with methylene chloride, washed with 0.1 N NaOH and reduced to dryness in vacuo. The residue was partitioned between 20% ethanol: cyclohexane and the steroids extracted from the aqueous layer with methylene chloride, dried, and chromatographed on a thin layer of Celite that had been impregnated with 0.8 g of formamide. After development in the butyl acetate: formamide: water (100:5:5, v/v) system, zones corresponding to aldosterone standards were eluted. In this system 18-hydroxycorticosterone migrated and was eluted with aldosterone.

The residue from the first chromatogram was extracted with methylene chloride, dried, dissolved in 0.2 ml of ethanol and 0.8 of 0.1 M periodic acid in 2% aqueous pyridine and permitted to stand overnight at room temperature. After oxidation of aldosterone and 18-hydroxycorticosterone to their corresponding etiolactones, as described by Ulick and Vetter (14), the procedure of Tait, Tait, Okamoto, and Flood (15) was then used for purification and quantitation of the etiolactones of aldosterone and 18-hydroxycorticosterone by extraction with methylene chloride, washing with sodium bicarbonate and development in a Bush-type paper chromatographic system, cyclohexane: benzene: methanol: water (5:3:5:1). The paper was then sprayed with blue tetrazolium solution in sodium hydroxide and heated gently for development of soda fluorescence. A Turner photofluorometer, which had been adapted for continuous automatic scanning of soda fluorescence, was used for quantitation. The sensitivity of the method is 0.05 μg with approximately 70% recovery for both steroids (checked by initial addition to the plasma of 1,2-³H-aldosterone).

RESULTS

1) Effect of angiotensin infusion on aldosterone secretory rate in primary and secondary aldosteronism. The patients with primary aldosteronism had a slightly lower control aldosterone secretory rate (291 ±25 μg/24 hr) than did the patients with secondary aldosteronism (362 ±90 μg/24 hr). After angiotensin infusion, aldosterone secretion increased markedly in the patients with primary aldosteronism with the resultant mean postangiotensin value of 561 μg ±113 μg/24 hr. On the other hand, the over-all effect of angiotensin infusion in secondary aldosteronism was a slight decrease in aldosterone secretioin to 336 ±56 μg/24 hr.

(2) Effect of angiotensin on the secretion of aldosterone by aldosterone-producing adenomas and nontumorous adrenal glands in vivo. The data pertinent to the patients studied are shown in Table I. Plasma renin activity and aldosterone secretory rate were normal in the patient with essential hypertension and appropriately elevated in the patients with secondary aldosteronism. Plasma renin activity in the anephric patient was 0. After demonstrating that the patients with primary aldosteronism had the characteristic findings of a high aldosterone secretory rate (364 μg/24 hr) and low upright plasma renin activity (74 ng/100 ml), these seven patients were then given spironolactone in a dose of 400 mg/day for at least 5 wk before further study. As a consequence, their endogenous renin-angiotensin system was activated and at the time of adrenal vein catheterization plasma renin activity had increased to 613 ng/100 ml and aldosterone secretory rate had increased to 536 μg/24 hr.

The result of short-term angiotensin infusion on the steroidal content of the adrenal venous effluent in these patients is demonstrated in Figs. 1–3. In all patients, except those with aldosterone-producing adenomas, there was little difference in the content of aldosterone and 18-hydroxycorticosterone emanating from the left and right adrenal gland. For this reason, values indicated for each patient represent an average of the steroid efflux from both adrenals.

In the patient with essential hypertension (Fig. 1), control aldosterone averaged 1.2 μg/100 ml, and following angiotensin, had fallen to 1.0 μg/100 ml. 18-Hydroxycorticosterone was 0.9 μg/100 ml before angiotensin and 1.5 μg/100 ml after angiotensin.

In the two patients with secondary aldosteronism (Fig. 1), the baseline concentration of aldosterone of the adrenal venous effluent averaged 0.5 and 1.0 μg/100

TABLE I

Base Line Data on Patients Studied during Adrenal Vein Catheterization

	PRA	ASR
	ng/100 ml	*μg/24 hr*
Essential hypertension	160	135
Secondary aldosteronism		
Patient 1	400	300
Patient 2	375	286
Anephric	0	–
Primary aldosteronism (7)		
Control		
Mean	71	364
Range	(48–100)	(167–687)
Spironolactone		
Mean	613	536
Range	(500–695)	(257–1200)

ml. After angiotensin infusion, there was either no change or an insignificant increase in the concentration of aldosterone in the effluent. Concentration of 18-hydroxycorticosterone exhibited a wider variation of base line values with a range from 1.0 to 3.4 μg/100 ml. After the angiotensin infusion, there was a slight increase in one patient but a modest fall in the other patient in the concentration of this steroid in the adrenal venous effluent.

In the anephric patient (Fig. 1), base line adrenal venous aldosterone and 18-hydroxycorticosterone was 0.53 and 2.05 μg/100 ml, respectively. After angiotensin infusion, aldosterone increased to 1.21 μg/100 ml, whereas 18-hydroxycorticosterone increased to 2.64 μg/100 ml.

In six of the seven patients with primary aldosteronism (Fig. 2), base line aldosterone for the nontumorous gland ranged between 0.4 and 0.8 μg/100 ml. The remaining patient had a base line aldosterone of 3.5 μg/100 ml. The mean for the group was 1.1 μg/100 ml. After angiotensin infusion, there was a modest increase in aldosterone content of the effluent resulting in a post-infusion value that averaged 1.7 μg/100 ml for the group.

Aldosterone content of the adrenal venous effluent from the adrenal gland harboring the aldosterone-producing adenoma was significantly higher than from the nontumorous glands of the same patient and ranged between 2.6 and 10.3 μg/100 ml, averaging 5.2 μg/100 ml. Although there was some overlap between the highest value seen for a nontumorous gland and the lowest observed in the effluent from an aldosterone-producing adenoma, there was no problem in distinguishing the tumorous from the nontumorous gland in any given patient.

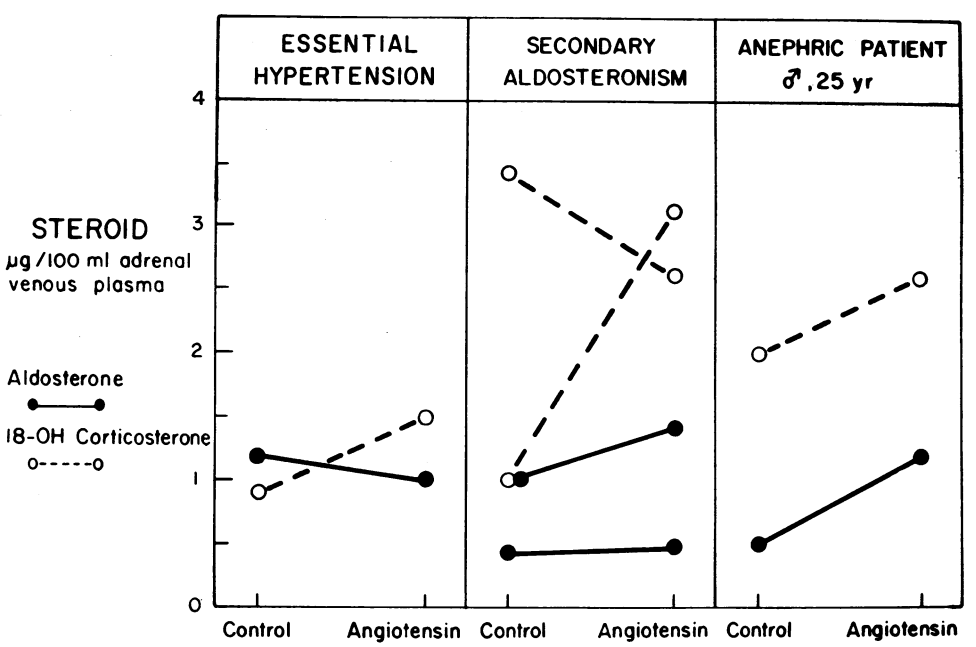

FIGURE 1 Effect of 10 min sub-pressor infusion of angiotensin on the adrenal venous content of aldosterone and 18-hydroxycorticosterone in a patient with essential hypertension, patients with secondary aldosteronism and an anephric patient. Note, scale extends only from 0–4 μg/100 ml.

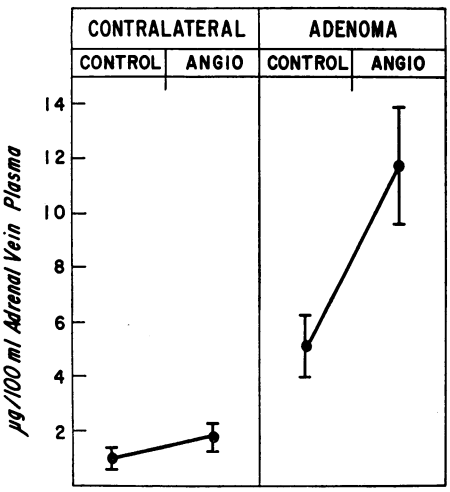

FIGURE 2 Effect of 10 min sub-pressor infusion of angiotensin on the adrenal venous concentration of aldosterone from the adenomatous and contralateral adrenals in seven patients with aldosterone-producing adenomas. Solid circles indicate means and bracketed horizontal lines indicate SEM.

After angiotensin infusion, there was a prompt rise in the aldosterone content in the adrenal venous effluent from the adrenal harboring the aldosterone-producing adenoma in six of the seven patients studied. Aldosterone concentration after angiotensin ranged between 5.8 and 20.4 μg/100 ml. One patient whose base line aldosterone was extraordinarily high (10.3 μg/100 ml) only demonstrated an increase of 0.5 μg/100 ml after angiotensin. However, after angiotension the mean adrenal venous aldosterone for the group was 11.9 μg/100 ml, for an average increase of 6.7 μg/100 ml.

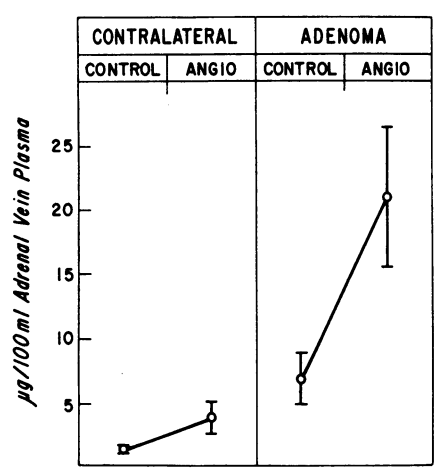

FIGURE 3 Effect of 10 min sub-pressor infusion of angiotensin on the adrenal venous concentration of 18-hydroxycorticosterone from the adenomatous and contralateral adrenals in seven patients with aldosterone-producing adenomas. Open circles indicate means and bracketed horizontal lines indicate SEM.

Similarly, base line 18-hydroxycorticosterone concentration from the nontumorous glands of patients with primary aldosteronism was low averaging 1.2 μg/100 ml (Fig. 3). The increase in 18-hydroxycorticosterone after short-term angiotensin infusion was somewhat greater than that observed for aldosterone and averaged 2.5 μg/100 ml for the group. As was the case with the base line aldosterone values, the concentration of 18-hydroxycorticosterone was always higher in the adrenal harboring the aldosterone-producing adenoma than in the nontumorous gland. 18-Hydroxycortisterone in the effluent from the tumorous glands ranged between 2.9 and 13.7 μg/100 ml and averaged 7.9 μg/100 ml. After angiotensin, 18-hydroxycorticosterone concentration in the effluent from the adrenal harboring the aldosterone-producing adenoma increased strikingly with values ranging between 9.3 and 47.2 μg/100 ml, for an average postangiotensin value of 20.1 μg/100 ml, an increase of 12.2 μg/100 ml over base line.

Sensitivity to infused angiotensin could be demonstrated in aldosterone-producing adenomas of all sizes (Fig. 4). Neither the base line level of aldosterone nor the increase in the steroid after angiotensin could be related to the size of the adenoma. Indeed, the patient with the second largest adenoma had the lowest base line value for aldosterone and one of the patients with the smallest tumor had one of the largest absolute increases in aldosterone concentration after angiotensin infusion.

(3) Effect of intravenous ACTH on the secretion of aldosterone from aldosterone-producing adenomas and nontumorous adrenals in vivo (*Table II*). Adrenal venous aldosterone was 0.5 μg/100 ml from the nonadenomatous adrenal and 4.85 μg/100 ml from the adrenal harboring the APA. After angiotensin aldosterone concentration in the effluent from the tumorous adrenal increased to 16.2 μg/100 ml and aldosterone concentration from the nontumorous adrenal increased slightly to 1.18 μg/100 ml.

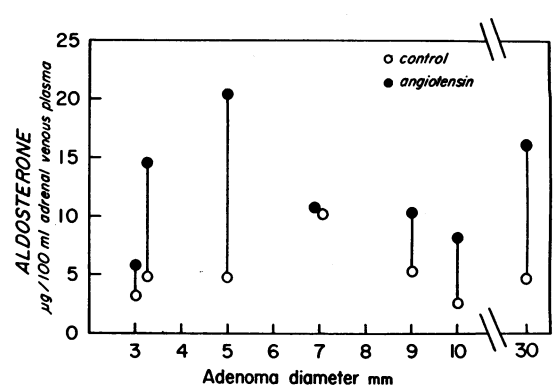

FIGURE 4 Relationship of adenoma size to concentration of aldosterone in the adrenal venous effluent before (open circles) and after (solid circles) 10 min angiotensin infusion.

TABLE II

Effect of Angiotensin and ACTH on the Adrenal Venous Efflux of Aldosterone in a Patient with an Aldosterone-Producing Adenoma

	Aldosterone
	μg/100 ml
Contralateral	
Control	0.5
Angiotensin	1.18
ACTH	5.7
Adenoma	
Control	4.85
Angiotensin	16.2
ACTH	14.0

After β1-24 ACTH, adrenal venous aldosterone from the nontumorous adrenal increased almost fivefold to 5.70 μg/100 ml above the maximum level achieved with angiotensin, whereas adrenal venous aldosterone from the tumor bearing adrenal was not further augmented and was 14.0 μg/100 ml, slightly below the level achieved with angiotensin.

DISCUSSION

Angiotensin has been clearly demonstrated to be a potent stimulus to aldosterone secretion. Its exact locus of action within the adrenal is not yet defined but increases in production of aldosterone and its precursors, 18-hydroxycorticosterone and corticosterone, may be demonstrated following either activation of the endogenous renin-angiotensin system (16), intravenous infusion of angiotensin (17, 18), or incubation of adrenal tissue with angiotensin (19). However, the normal adrenal does not have an unlimited capacity to respond to progressive increases in plasma angiotensin with further increases in aldosterone secretion. Ames, Borkouski, Sicinski, and Laragh (20) have shown that in normal man maximal aldosterone secretion is achieved when angiotensin is infused at a rate of 0.5 μg/min. Further increases in the rate of angiotensin infusion are not accompanied by further increases in aldosterone secretion. Furthermore, Cannon, Ames, and Laragh (21) have demonstrated that the aldosterone stimulatory effect of infused angiotensin is dependent upon the state of activation of the endogenous renin-angiotensin system at the time of the infusion. When endogenous plasma renin activity is suppressed after a high salt diet, angiotensin infusion evokes a relatively greater increase in aldosterone secretion than when endogenous plasma renin activity is increased as it is following a low salt diet.

In view of this, the differential effect of angiotensin infusion on 24-hr aldosterone secretory rate in the patients with primary and secondary aldosteronism might be explained on the basis of differences in the activity of the renin-angiotensin system at the time of the infusion. Thus, the patients with secondary aldosteronism could not respond to infused angiotensin with further increases in aldosterone secretion because the zona glomerulosa of both adrenals was already maximally responding to elevated levels of endogenously generated angiotensin (22).

However, in the patients with primary aldosteronism, under control conditions virtually all of the aldosterone secretion emanates from the aldosterone-producing adenoma. Plasma renin activity is depressed and as a result normal zona glomerulosa is unstimulated. Under these circumstances, the increase in aldosterone secretion observed after angiotensin infusion may represent new steroidogenesis in the previously unstimulated zona glomerulosa and not indicate further secretion of aldosterone from the adenoma.

The data gleaned from the adrenal vein studies argue against this point. For one, it is clear that under the conditions of the study angiotensin infusion evoked marked increases in aldosterone secretion from the adenoma and only trivial changes in aldosterone secretion from nontumorous tissue. Secondly, the level of plasma renin activity alone was not a factor, for in the anephric patient whose plasma renin activity was 0, angiotensin infusion did not result in any significant change in aldosterone secretion. Furthermore, artificial elevation of plasma renin activity in patients with primary aldosteronism by the use of spironolactone did not blunt the sensitivity of the aldosterone-producing adenoma to infused angiotensin.

The observations on the secretion of 18-hydroxycorticosterone are essentially in agreement with the findings of Ulick, and Vetter and Ulick, Nicolis, and Vetter (14, 16). For any single adrenal, the production of 18-hydroxycorticosterone always exceeded that of aldosterone. This was true of both tumorous and nontumorous tissue.

As suspected the increased base line secretion of 18-hydroxycorticosterone seen in primary aldosteronism (16) was due to excess secretion of this hormone by the aldosterone-producing adenoma.

In addition, it has been possible to demonstrate that short-term infusions of angiotensin in doses that were incapable of producing significant increases in aldosterone secretion in nontumorous adrenals were capable of producing modest but definite increases in secretion of 18-hydroxycorticosterone in 9 of 11 nontumorous adrenals. Whether this means that preformed 18-hydroxycorticosterone must be available before aldosterone secretion can increase after angiotension is not clear from these studies. It is of interest, however, that in the efflux from the aldosterone-producing adenoma after angiotensin the absolute increase in 18-hydroxycorti-

costerone secretion was more than twice that observed for aldosterone.

In vitro studies have demonstrated that aldosterone-producing adenomas contain amounts of aldosterone that are far in excess of these seen in nontumorous tissues (23–26). The current study adds further support to the concept that the aldosterone-producing adenoma secretes large amounts of aldosterone and does so in response to doses of angiotensin that are incapable of evoking any change in aldosterone secretion in nontumorous adrenals. These data suggest that the aldosterone-producing adenoma is more sensitive than normal adrenal tissue to small changes in plasma renin activity.

However, there are several studies which demonstrate that those maneuvers that affect the aldosterone secretory rate, by virtue of their ability to stimulate or depress plasma renin activity in normals, do not affect aldosterone secretory rate in patients with primary aldosteronism. Bartter and Biglieri (27), and Biglieri and Forsham (28), have shown that low salt and high salt diets do not affect aldosterone excretion in patients with primary aldosteronism. Espiner, Tucci, Jagger, and Lauler (29) have reported that in normal subjects who have had secondary aldosteronism induced by a low salt diet, volume expansion with saline infusion resulted in a return to normal aldosterone secretory rate, whereas in patients with primary aldosteronism, aldosterone secretory rate remains virtually unchanged after saline infusion. Biglieri, Slaton, Kronfield, and Schambelan (30) have reported that while expansion of intravascular volume by desoxycorticosterone acetate (DOCA) administration decreases aldosterone secretory rate in normal patients and those with secondary aldosteronism, it does not affect aldosterone secretory rate in primary aldosteronism.

These data appear to be incompatible with the thesis that the aldosterone-producing adenoma is responsive to changes in plasma renin activity. However, the point at issue may be not the failure of the aldosterone-producing adenoma to respond to changes in plasma renin activity, but rather that plasma renin activity has not been altered in the first place.

Maneuvers that expand intravascular volume and thereby decrease plasma renin activity in normal patients may not achieve the same effect in patients with aldosterone-producing adenomas. Although normal patients receiving saline infusion and DOCA respond to these experimental manipulations by significant weight gain, for some reason the patients with primary aldosteronism either had no significant change in weight or an actual decrease in weight after these maneuvers (29, 30).

When plasma volume is convincingly altered, plasma renin activity does appear to be activated and produces appropriate changes in aldosterone secretory rate. Thus, Biglieri and Forsham (27) have shown that in patients with primary aldosteronism, phlebotomy of 400 ml of blood results in marked increases in aldosterone secretion, whereas Plasmanate infusion substantially decreases aldosterone secretory rate. Similarly, Muller, Manning, and Hodler (31) have shown that whereas changes in dietary sodium are ineffective in altering aldosterone excretion in primary aldosteronism, infusion of 70 g of albumin results in a 50% reduction in aldosterone excretion in these patients. It would appear then that the observed differences between the studies are related to the effectiveness of the proposed maneuvers to alter plasma renin activity in primary aldosteronism, rather than the inability of the adrenal adenoma to respond to fluctuations in plasma renin activity. Regardless of the mechanism involved, procedures such as saline infusion in DOCA administration remain useful diagnostic tests to distinguish patients with secondary aldosteronism from those with primary aldosteronism (32).

If the aldosterone-producing adenoma is sensitive to small changes in plasma renin activity and if plasma renin activity does increase when assuming the upright posture, then one would expect that aldosterone excretion in primary aldosteronism should exhibit some diurnal variation. The data on this point are at variance. Although one study has demonstrated a normal diurnal excretion of tetrahydroaldosterone (33), other studies show either no consistent pattern or a reversal of normal diurnal pattern in excretion of the metabolite of aldosterone hydrolyzed at pH 1 (28). These differences may be difficult to resolve for the results of aldosterone excretion tend to be expressed in terms of μg/12 hr time period rather than per standard volume. In primary aldosteronism, urinary volume during upright hours is so low (34) that measurement of aldosterone in the urine of these hyposthenuric patients may not be an accurate reflection of secretion of aldosterone into the blood.

Recent studies utilizing the technique of Brodie, Melby, Tait, and Tait (35) for measuring plasma aldosterone concentration have demonstrated that plasma aldosterone concentration is high during recumbency in patients with primary aldosteronism and rises strikingly after 4 hr of upright posture despite only minimal increases in plasma renin activity (36).

One unresolved paradox is the fact that although short-term infusion of angiotensin resulted in marked increases in aldosterone secretion from the APA, chronic elevation of endogenous PRA did not result in *pari passu* increases in aldosterone secretion. Thus after spironolactone therapy while PRA increased from 71 to 613 ng/100 ml, ASR increased only from 364 to 536 μg/24 hr. One cannot invoke the concept that the adenoma had reached a level of maximum steroid output after increases in PRA, for at this level of PRA, small increments of infused angiotensin produced large increases in aldosterone efflux. It probably does not repre-

sent any difference in responsiveness to infused or endogenously generated angiotensin, for the same phenomenon of increased responsiveness to endogenously generated angiotensin has been demonstrated in patients with APA's after 4 hr in the upright position (36).

The observed discrepancy probably reflects a differential response to acute vs, chronic elevations of plasma angiotensin. It appears that with artificially induced elevations of plasma renin activity a new steady state is created vis à vis the APA. Chronic elevations of PRA produce substantial but not fully comparable increases in ASR. Still, at this new steady state, small increments in angiotensin continue to evoke large increases in aldosterone secretion.

The role of ACTH in the regulation of aldosterone secretion is controversial. Initial studies indicated that ACTH was not important in the control of aldosterone secretion (37, 38). Subsequent studies have demonstrated that whereas aldosterone is still secreted in the absence of ACTH, secretion and responsiveness to alterations in the activity of the renin-angiotensin system is diminished (39–41).

ACTH infusion appears to induce a definite but transient increase in aldosterone secretion (42). As was the case with angiotensin infusion, the response to infused or injected ACTH appears to be dependent on the state of activation of the renin-angiotensin system at the time of the infusion. However, unlike angiotensin, ACTH is maximally effective as a stimulus to aldosterone secretion when PRA is elevated as it is following low salt diet, and least effective when PRA is suppressed as it is following salt loading (42, 43).

Studies on the role of ACTH in primary aldosteronism are scant. A syndrome has been described in which hyperaldosteronism and hypertension are presumed secondary to increased levels of ACTH (44) but these patients are not thought to have adenomas.

In those patients with proven APA's the response to ACTH does not appear to differ from the response observed in normals. Thus, repeated daily injections of ACTH produce only a transient rise in aldosterone secretion (45).

The results of the present study indicate that while the nontumorous adrenal responded to ACTH with a five-fold increase in aldosterone above the angiotensin stimulated level, the adrenal harboring the APA was not capable of further augmenting of aldosterone secretion above the level achieved with angiotensin.

This suggests that the transient increase in aldosterone secretion seen after ACTH in patients with APA's may represent new steroidogenesis in the nontumorous adrenal. This is the converse of what was observed following angiotensin.

It must be stressed that none of the patients studied in this series was a truly normal control. The current techniques of adrenal vein catheterization that involve extensive time under the fluoroscope preclude the study of normal volunteers at this time. Therefore, the observations reported are confined to differences between aldosterone-producing adenomas and nontumorous adrenal tissue.

In summary, it appears that the aldosterone-producing adenoma is uniquely sensitive to small increases in plasma angiotensin and responds to these changes with unusually large increases in aldosterone secretion. This response is not observed with ACTH. These observations give rise to the speculation that there may be a metabolically unique population of cells, which by virtue of their exquisite sensitivity to angiotensin, are selected out to develop as the aldosterone-producing adenoma.

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