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Research Article

Studies were undertaken to determine what part of the aldosterone biosynthetic pathway is stimulated by angiotensin and potassium. The availability of a method for isolating the early portion of the aldosterone pathway and a new method for measuring plasma deoxycorticosterone permitted the design of experiments to determine whether angiotensin and potassium stimulate the pathway before deoxycorticosterone. To eliminate ACTH-dependent steroid synthesis, the experiments were performed in subjects receiving constant dosage of dexamethasone. To minimize the intra-adrenal conversion of deoxycorticosterone to corticosterone, all subjects also received constant dosage of metyrapone. Plasma deoxycortisol was measured as an index of the activity of the zona fasciculata. In the absence of changes in plasma deoxycortisol, one may infer that changes in plasma deoxycorticosterone represent changes in function of zona glomerulosa, the site of aldosterone formation. Under these conditions, human subjects responded both to angiotensin and to potassium with significant increases in plasma deoxycorticosterone but without significant increases in plasma deoxycortisol. In contrast, small doses of ACTH given under similar conditions never induced increases in plasma deoxycorticosterone without simultaneously inducing large increases in plasma deoxycortisol. It is concluded that the aldosterone-stimulating effects of angiotensin and potassium are, at least in part, consequences of stimulation of the biosynthetic pathway at some point before the formation of deoxycorticosterone so as to increase the availability of aldosterone precursors.

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Site of Stimulation of Aldosterone Biosynthesis by Angiotensin and Potassium

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ABSTRACT Studies were undertaken to determine what part of the aldosterone biosynthetic pathway is stimulated by angiotensin and potassium. The availability of a method for isolating the early portion of the aldosterone pathway and a new method for measuring plasma deoxycorticosterone permitted the design of experiments to determine whether angiotensin and potassium stimulate the pathway before deoxycorticosterone. To eliminate ACTH-dependent steroid synthesis, the experiments were performed in subjects receiving constant dosage of dexamethasone. To minimize the intra-adrenal conversion of deoxycorticosterone to corticosterone, all subjects also received constant dosage of metyrapone. Plasma deoxycortisol was measured as an index of the activity of the zona fasciculata. In the absence of changes in plasma deoxycortisol, one may infer that changes in plasma deoxycorticosterone represent changes in function of zona glomerulosa, the site of aldosterone formation. Under these conditions, human subjects responded both to angiotensin and to potassium with significant increases in plasma deoxycorticosterone but without significant increases in plasma deoxycortisol. In contrast, small doses of ACTH given under similar conditions never induced increases in plasma deoxycorticosterone without simultaneously inducing large increases in plasma deoxycortisol. It is concluded that the aldosterone-stimulating effects of angiotensin and potassium are, at least in part, consequences of stimulation of the biosynthetic pathway at some point before the formation of deoxycorticosterone so as to increase the availability of aldosterone precursors.

INTRODUCTION

Although it is well established (1–5) that angiotensin and potassium stimulate adrenal synthesis of aldosterone, comparatively little attention has been given to the intra-adrenal mechanism through which these agents effect increases in aldosterone synthesis. It has been established (6–11) that aldosterone is synthesized by a series of reactions that involve cholesterol, pregnenolone,[1] progesterone, deoxycorticosterone (DOC), corticosterone, and probably 18-hydroxycorticosterone. In the present investigation, we have taken advantage of the availability of methods for "isolating" in vivo the early part of the aldosterone pathway (before the formation of DOC) and the availability of a new method (12) for measuring plasma DOC in order to determine whether angiotensin and potassium act early in the pathway so as to stimulate the biogenesis of aldosterone precursors.

In studying the production of DOC by the zona glomerulosa, the site of aldosterone synthesis, one is confronted by two problems. First, most of the DOC formed by the adrenal is rapidly converted to corticosterone. In order to minimize this conversion and bring about the secretion of DOC, one can use the 11β-hydroxylase inhibitor, metyrapone. Accordingly, this drug was used as a constant condition throughout the control and experimental periods in the present study. Second, in addition to the DOC formed in the zona glomerulosa where it can serve as a precursor for aldosterone, there is a relatively large amount of DOC formed in the zona fasciculata under the influence of ACTH. In order to eliminate ACTH-dependent formation of DOC, one can suppress ACTH secretion by the administration of dexamethasone. Accordingly, this drug was also used as a constant condition throughout the control and experimental periods of the present study. In this way the early part of the aldosterone biosynthetic pathway was "isolated." As a

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[1] *Abbreviations and trivial names used in this paper*: deoxycortisol, 17, 21-dihydroxypregn-4-ene-3, 20-dione; deoxycortisol acetate, 17, 21-dihydroxy-3, 20-dioxopregn-4-en-21-yl acetate; DOC, deoxycorticosterone; 18-hydroxycorticosterone, 11β, 18, 21-trihydroxypregn-4-ene-3, 20-dione; pregnenolone, 3β-hydroxypregn-5-en-20-one; progesterone; pregn-4-ene-3, 20 dione.

measure of the success of this isolation, contemporary measurement of deoxycortisol was performed. This steroid is formed by the zona fasciculata under the influence of ACTH and is normally readily converted to cortisol; in the presence of metyrapone, however, it is released into the circulation and can serve as an index of ACTH-dependent adrenal activity.

METHODS

Clinical studies. Normal ambulatory subjects and a patient with an isolated ACTH deficiency were studied while on a 110 mEq sodium diet. To prevent the release of ACTH, subjects were given dexamethasone 0.375 mg every 4 hr for 2 days preceding and during the experimental period. Each subject was also given metyrapone 500 mg every 4 hr beginning 24 hr before and continuing throughout the experimental period. Before the start of the angiotensin infusion subjects were placed in recumbency for 2 hr. Angiotensin II (Hypertensin-CIBA, CIBA Pharmaceutical Company, Summit, N. J.) was infused at a rate (6–10 ng/kg per min) sufficient to elevate the blood pressure 15–20 mm Hg. Blood was sampled just before and at the end of the 30 min infusion. 24 hr after the angiotensin infusion and while subjects continued to take dexamethasone and metyrapone, potassium was administered. Subjects were given 30 mEq of potassium (KCl elixir) every 4 hr for 24 hr. The total potassium intake, including dietary sources, during this 24 hr period was 250 mEq. Blood samples were obtained just before and at the end of the potassium loading. Three subjects also were given 0.1 U ACTH as an intravenous infusion over a period of 1 hr to demonstrate the comparative effects of ACTH on plasma DOC and deoxycortisol under these experimental conditions.

Steroid analyses. Plasma DOC was measured by a competitive-binding technique as previously described (12). Plasma aldosterone was measured by a double isotope dilution method (13) and plasma cortisol was determined using the fluorometric technique (14).

Plasma deoxycortisol was measured in the same plasma sample used for the determination of DOC. Since the deoxycortisol method has not been previously reported it will be presented in some detail now.

Plasma deoxycortisol assay

Materials. Deoxycortisol-1,2-^{3}H (New England Nuclear Corp., Boston, Mass.), SA 35 Ci/mM, was purified by thin-layer chromatography. Purity was confirmed by finding constant specific activity when a sample was repeatedly recrystallized with authentic deoxycortisol. The deoxycortisol (Mann Research Labs, Inc., New York) had been recrystallized twice from ethanol and had a melting point of 214–216°C. Deoxycortisol acetate (Mann Research Labs, Inc., New York) recrystallized twice from methanol had a melting point of 238°C. Solvents, thin-layer chromatography plates, and corticosteroid-binding-globulin were prepared as previously described (12).

Method. Before extraction, 1000 dpm of deoxycortisol-^{3}H was added to each plasma sample to correct for procedural losses. Extraction and initial chromatography were the same as used in the DOC assay (12). Using cortisone acetate as a marker, the area on the plate corresponding to deoxycortisol was scraped off and the silica gel eluted using 1 ml of acetone. The eluate was dried under air and the residue dissolved in 5 drops of pyridine and acetylated overnight using 5 drops of acetic anhydride. After acetylation the samples were transferred to thin-layer plates and developed in an unsaturated ether : benzene (2 : 1) system. 1-amino-4-*N*-methylaminoanthraquinone, F_{II}, (K and K Laboratories, Inc., Plainview, N. Y.) was used as a marker. After chromatography the plates were dried *in vacuo* at 60°C for 15 min. The area on the plate corresponding to deoxycortisol acetate was removed and the silica gel eluted using 1.0 ml of 10% acetone in ether. Determination of tracer recovery and performance of the competitive-binding assay were carried out as previously described for DOC (12) except that deoxycortisol acetate was used to construct the standard curve.

Evaluation of the method. Both the water blank and the plasma blank were zero. Specificity was determined by assaying multiple 5-ml samples of a mixture of steroids, each present in a concentration equivalent to 20 μg/100 ml. There was no interference by these steroids (Table I). The method can detect as little as 0.2 ng. The intra-assay coefficient of variation, determined by assaying six 5-ml portions from a plasma pool containing 30 ng/100 ml of deoxycortisol, was 13%. Recovery of added deoxycortisol to

TABLE I

Steroids Proven Not to Interfere with the Deoxycortisol Assay

C-21 Steroids
3β-hydroxypregn-5-en-20 one
3β, 17-dihydroxy-pregn-5-en-20-one
3β, 21-dihydroxy-pregn-5-en-20-one
pregn-4-ene-3, 20-dione
6β-hydroxypregn-4-ene-3, 20-dione
11β-hydroxypregn-4-ene-3, 20-dione
pregn-4-ene-3, 11, 20-trione
14-hydroxypregn-4-ene-3, 20-dione
17-hydroxypregn-4-ene-3, 20-dione
20α-hydroxypregn-4-en-3-3one
20β-hydroxypregn-4-en-3-one
21-hydroxypregn-4-ene-3, 20-dione
11β, 17-dihydroxypregn-4-ene-3, 20-dione
11β, 17, 21-trihydroxypregn-4-ene-3, 20-dione
17, 21-dihydroxypregn-4-ene-3, 11, 20-trione
11β, 21-dihydroxypregn-4-ene-3, 20-dione
11β, 21-dihydroxy-3, 20-dioxopregn-4-en-18-al
21-hydroxypregn-4-ene-3, 11, 20-trione
5β-pregnane-3α, 20α-diol
5β-pregnane-3α, 17, 20α-triol
3α, 11β, 17, 21-tetrahydroxy-5β-pregnan-20-one
C-19 Steroids
androst-4-ene-3, 17-dione
androst-5-ene-3β, 17-diol
3β-hydroxyandrost-5-en-17-one
3α-hydroxy-5α-androstan-17-one
17β-hydroxyandrost-4-en-3-one
3β, 17β-dihydroxyodrost-4-en-3-one
17-oxoandrost-5-en-3β-yl sulfate
C-18 Steroids
3-hydroxyestra-1, 3, 5, (10)-trien-17-one
estra-1, 3, 5, (10)-triene-3, 17β-diol
estra-1, 3, 5, (10)-triene-3, 16α, 17β-triol

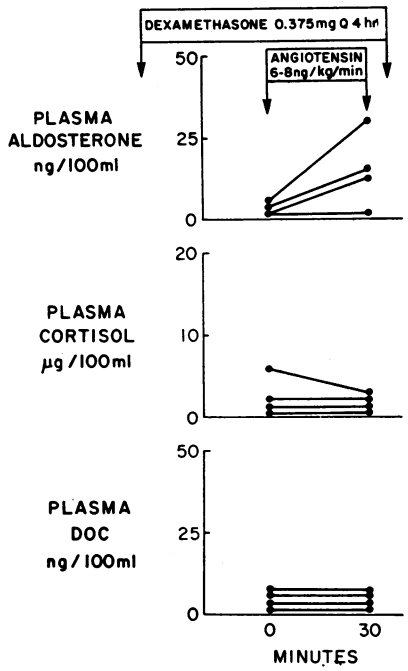

FIGURE 1 Effects of angiotensin on plasma aldosterone, cortisol, and DOC concentrations; angiotensin was infused intravenously for 30 min into normal subjects who were given dexamethasone (0.375 mg every 4 hr) to prevent ACTH secretion.

water, after correction for procedural losses was 97±13% (mean ±SD). 11 normal subjects were found to have 8 a.m. plasma levels ranging from 8 to 66 ng/100 ml (mean = 32 ng/100 ml).

RESULTS

Effect of angiotensin on plasma aldosterone, cortisol, and DOC. A preliminary experiment was performed to assure that, under the conditions employed, the amount of angiotensin infused was sufficient to increase plasma aldosterone but not to increase plasma cortisol. The effect on plasma DOC was also determined. Four subjects, while receiving dexamethasone but not metyrapone, were infused with angiotensin for 30 min. As noted in Fig. 1, three of the four subjects had distinct elevations of plasma aldosterone. Within the limits of the sensitivity of these asays, however, no effect of angiotensin on plasma cortisol or DOC was detected.

Effect of the angiotensin on plasma DOC and deoxycortisol of subjects receiving dexamethasone and metyrapone. Angiotensin was infused for 30 min while subjects were receiving constant amounts of dexamethasone and metyrapone. As depicted in Fig. 2, plasma DOC rose distinctly in all subjects except one. In contrast, there was no consistent change in plasma deoxycortisol.

Effect of potassium on plasma DOC and deoxycortisol of subjects receiving dexamethasone and metyrapone.

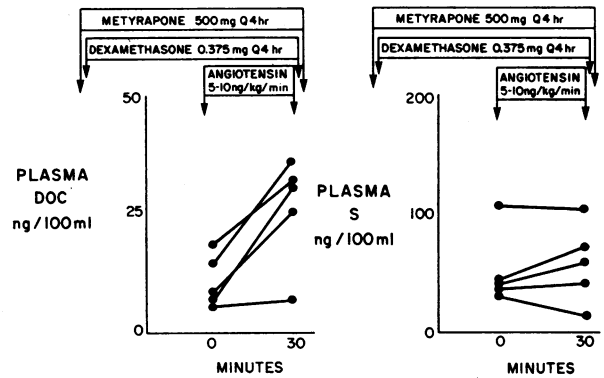

FIGURE 2 Effects of angiotensin on plasma DOC and deoxycortisol (S) concentrations when infused for 30 min into normal subjects receiving both dexamethasone and metyrapone.

After potassium administration, serum potassium concentrations rose 0.45±0.04 (mean ±SD) mEq over base line levels. Plasma DOC rose in all five subjects, while no significant change was noted in plasma deoxycortisol (Fig. 3).

Studies of a patient with isolated ACTH deficiency. Fig. 4 summarizes the results of a series of studies on a patient with ACTH deficiency, employing the protocol used for the normal subjects. In addition, the effect of sodium restriction on plasma DOC and deoxycortisol was determined in this subject. It had previously been shown that his aldosterone secretion rate increased after sodium depletion. Angiotensin, sodium restriction, and potassium loading each caused increases in plasma DOC in contrast to the lack of a significant effect on plasma deoxycortisol.

Effects of low doses of ACTH. This experiment was performed to rule out the possibility that the DOC secreted in response to angiotensin and potassium came from corticosterone-producing cells located in the zona

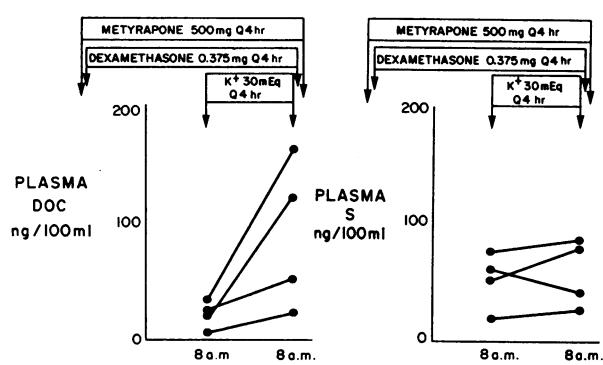

FIGURE 3 Effects of potassium on plasma DOC and deoxycortisol (S) concentrations. Potassium chloride was administered orally for 24 hr to normal subjects receiving both dexamethasone and metyrapone.

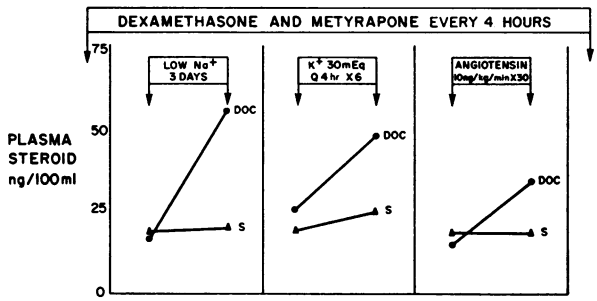

FIGURE 4 Effects of low sodium diet, potassium, and angiotensin on plasma DOC and deoxycortisol (S) in a patient with isolated ACTH deficiency receiving constant doses of dexamethasone and metyrapone.

fasciculata and also to exclude the possibility that the effects of angiotensin and potassium were mediated through ACTH. Subjects were given dexamethasone and metyrapone as before and then infused with small doses of ACTH. In Fig. 5 the response of the adrenal gland to this stimulus is compared to the response after potassium administration. Although ACTH caused increases in both DOC and deoxycortisol, potassium caused increases only in plasma DOC.

DISCUSSION

It is generally thought that all hormonal steroids are derived from cholesterol, and it has been shown that in some cases the rate-limiting step in steroidogenesis is in the conversion of cholesterol to pregnenolone. Although this might also be true for aldosterone, there is a paucity of data bearing on the question of where the biosynthetic pathway of this steroid is affected by the agents that stimulate its secretion under various physiologic conditions. Bledsoe, Island, and Liddle (15) have adduced that sodium depletion stimulates the early part of the aldosterone biosynthetic pathway, before the formation of DOC. Although sodium depletion might have acted through elevating angiotensin levels, it might alternatively have acted through some other mechanism, since it has been shown that a decrease in the concentration of sodium ion in blood perfusing the adrenal can also stimulate aldosterone secretion (16). Therefore, in the present study the effect of angiotensin itself was examined, while sodium intake was held constant. In other experiments potassium was administered, and in still others small doses of ACTH were administered in order to ascertain their effects on plasma steroid levels. Under the experimental conditions that were employed, angiotensin and potassium both appeared to stimulate the pre-DOC portion of the aldosterone biosynthetic pathway without appreciably affecting the cortisol biosynthetic pathway. In contrast, small doses of ACTH did not selectively stimulate the aldosterone pathway. When ACTH stimulated DOC production, it invariably stimulated the cortisol pathway. It is inferred from this that angiotensin and potassium stimulated DOC secretion directly, not through stimulating ACTH secretion.

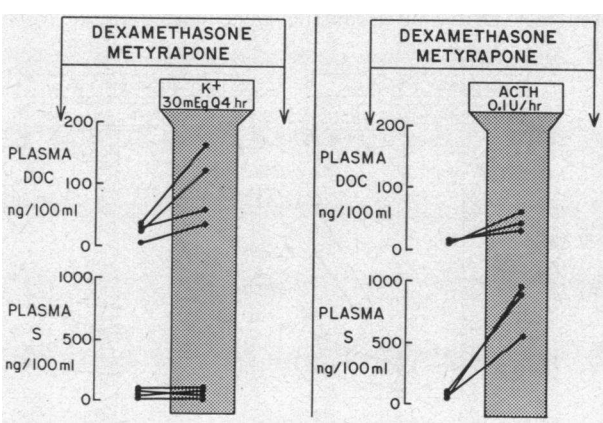

FIGURE 5 Comparative effects of potassium and ACTH on plasma DOC and deoxycortisol (S) levels in normal subjects receiving constant doses of dexamethasone and metyrapone.

The acute effect of ACTH is to stimulate the conversion of cholesterol to pregnenolone. Cells of the zona fasciculata convert pregnenolone to both cortisol and corticosterone; but the zona glomerulosa, lacking 17α-hydroxylase, converts pregnenolone to corticosterone then to aldosterone (17). Our observations that angiotensin and potassium selectively stimulated DOC production without stimulating deoxycortisol secretion are consistent with the view that in these experiments these agents acted mainly on the zona glomerulosa. The observation that ACTH stimulated both DOC and deoxycortisol secretion is consistent either with the view that it acted only on the zona fasciculata or that it acted both on the zona fasciculata and the zona glomerulosa. In any event, the adrenal responses to potassium and angiotensin were clearly different from the responses to ACTH. The specificity of our responses in man differs from the results of certain experiments in dogs in which both sodium depletion and angiotensin appeared to stimulate the zona fasciculata (18, 19).

In order to obtain information about the *early* part of the aldosterone biosynthetic pathway, it is necessary to impose some limitation on the facility with which adrenal enzymes can transform steroid intermediates to hormonal steroids. In the present study, metyrapone was used to prevent DOC from being converted to corticosterone and then to aldosterone. The methodological importance of using metyrapone is illustrated by comparing Fig. 1 with Fig. 2. In the absence of metyrapone, angiotensin increases aldosterone secretion without appreciably affecting the release of DOC into the circulation. This is

in agreement with a recent report by Rosen, Laidlaw, and Ruse (20). However, in the presence of metyrapone, aldosterone synthesis is inhibited at the level of DOC utilization (21), and the administration of angiotensin brings about a distinct increase in DOC secretion.

In order to obtain clearcut results in clinical studies of this type it is necessary to eliminate insofar as possible the production of deoxycorticosterone by cells of the zona fasciculata, which are not involved in aldosterone synthesis, but which in the presence of metyrapone respond to ACTH by secreting large quantities of DOC. This superabundance of DOC, which is irrelevant to the present problem, would make it difficult to discern with precision the relatively small quantities of DOC elaborated by aldosterone-secreting cells of the zona glomerulosa. This problem is solved by suppressing ACTH secretion and thus reducing zona fasciculata function to a minimum. That ACTH-dependent adrenal function was indeed suppressed can be judged from the fact that plasma deoxycortisol concentrations were only about 50 ng/100 ml in our subjects receiving metyrapone and dexamethasone, where as in normal subjects receiving metyrapone without dexamethasone, plasma deoxycortisol concentrations range from 3,000 to 20,000 ng/100 ml (22). In normal subjects receiving neither metyrapone nor dexamethasone plasma cortisol concentrations range from 5,000 to 25,000 ng/100 ml (14).

We conclude that angiotensin, sodium depletion, and potassium act, at least in part, early in the aldosterone biosynthetic pathway, before the formation of DOC. These findings are in accord with results of in vitro experiments (23–25) suggesting that the site of action of angiotensin and potassium is early in the aldosterone biosynthetic pathway, probably before the formation of pregnenolone. At what point in the steroidogenic pathway the various stimuli converge to produce their common effect of accelerating the formation of DOC remains to be determined. Our data do not, of course, exclude the possibility that these stimuli also act late in the pathway, increasing the conversion of corticosterone to aldosterone (26, 27).

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