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NEW MINERALCORTICOIDS IN THE SYNDROME OF LOW-RENIN ESSENTIAL HYPERTENSION

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SUMMARY

Patients with low-renin essential hypertension have certain features consistent with excessive mineralocorticoid activity. Because known mineralocorticoids are normal in the majority of low-renin essential hypertension patients, an unknown mineralocorticoid was sought in the urine of such patients. Using adrenalectomized rats to assay mineralocorticoids, urine extracts from patients with low-renin essential hypertension were found to contain more mineralocorticoid activity than could be accounted for by the known mineralocorticoids in the extract. The substances causing this unexplained mineralocorticoid activity were purified and then identified by mass spectral analysis as 16β-hydroxydehydroepiandrosterone (16β-OH-DHEA) and its 16-oxo-17β-ol isomer. That these steroids are in fact mineralocorticoids was confirmed by demonstrating that the synthetic compounds have mineralocorticoid potency 1/40th that of aldosterone in the rat bioassay. Also, the mineralocorticoid effects of both the urine extracts and the synthetic steroids were blocked in the rat by spironolactone, a mineralocorticoid antagonist. The new mineralocorticoids appear to have clinical significance since they appeared in the urine in abnormally large quantities in 15 out of 15 patients with low-renin essential hypertension but only one out of 14 patients with hypertension and normal or high plasma renin activity.

INTRODUCTION

The phenomenon of "low renin hypertension" began to take shape in 1963 when the group at St. Mary's Hospital in London announced that plasma renin activity (PRA) was subnormal in patients with primary aldosteronism [1]. When it was realized that this feature could be used to distinguish primary aldosteronism from secondary aldosteronism, a combination of subnormal PRA and supernormal aldosterone became the diagnostic *sine qua non* of primary aldosteronism [2].

It soon became apparent, however, that primary aldosteronism was not the only disorder characterized by subnormal PRA. Licorice intoxication [3], excessive ingestion of sodium [4], hypersecretion of 11-deoxycorticosterone [5], and hypersecretion of 18-hydroxy-11-deoxycorticosterone [6] have all been shown to be associated with hypertension and subnormal PRA. Furthermore, fully 20 per cent of patients with essential hypertension were also found to have very low PRA values [7]. Thus the syndrome of "low renin essential hypertension" came into being.

In 1966 we postulated that the syndrome of low renin essential hypertension might reflect an excess of some mineralocorticoid. When measurements of the known mineralocorticoids yielded only normal results in several patients, we postulated that there might be an excess of some unknown mineralocorticoid.

As an initial test of this hypothesis, we performed a double blind study of the antihypertensive efficacy of amino-glutethimide, a drug that inhibits the first step in the biosynthesis of all steroids, the conversion of cholesterol to pregnenolone [8]. We observed a decrease in blood pressure of patients with low-renin essential hypertension but not of patients with normal-renin essential hypertension [9]. On the basis of these observations, we and others were encouraged to look for still further evidence that low-renin essential hypertension might be caused by an excess of some unidentified mineralocorticoid.

The next stage in the evolution of the hypothesis was the therapeutic trial of the mineralocorticoid antagonist, spironolactone, as an antihypertensive agent, to ascertain whether it would help discriminate between low-renin essential hypertension and normal-renin essential hypertension. Three groups [10–12], including our own, performed controlled studies, and all concluded that spironolactone was significantly more efficacious in patients with low-renin essential hypertension than in patients with essential hypertension and normal plasma renin activity. Thus, once again, patients with low-renin essential hypertension behaved *as though* they had an excess of some mineralocorticoid even though in most cases direct assays of the known mineralocorticoids had failed to demonstrate such an excess.

In 1973, we began a search for a new mineralocorticoid in the urine of patients with low-renin essential hypertension. First, a method for assaying mineralocorticoid activity was developed utilizing adrenalectomized rats as assay subjects. A decrease in the urinary sodium: potassium ratio was used as an index of mineralocorticoid activity. Aldosterone was employed as a standard.

In an initial set of experiments, urine was extracted with dichloromethane without prior hydrolysis of steroid conjugates. The dichloromethane extracts were assayed for mineralocorticoid activity, and extracts of urine obtained from patients with low-renin essential hypertension were found to have more

mineralocorticoid activity than did extracts of urine obtained either from normal subjects or from patients with essential hypertension and normal plasma renin activity. The urine extracts were then assayed for aldosterone, 11-deoxycorticosterone, corticosterone, and cortisol by specific radioimmunoassay or physicochemical assay methods. The quantities of these known steroids that were actually found in the extracts were then used in the mineralocorticoid bioassay. In studies of urine from patients with low-renin essential hypertension, the known steroids were significantly less effective in the mineralocorticoid bioassay than were the crude extracts themselves, but such large disparities between "expected" and "observed" mineralocorticoid activity were not found in extracts of urine from other subjects. This was taken as evidence that the urine of patients with low-renin essential hypertension contained a mineralocorticoid other than aldosterone, 11-deoxycorticosterone, corticosterone, and cortisol.

Using the bioassay in combination with various solvent fractionation procedures and thin layer chromatography, an attempt was then made to separate the major biologically active component from all of the known mineralocorticoids. This was ultimately successful, and the "purified" unknown mineralocorticoid was then studied using gas–liquid chromatography and mass spectrometry.

To our surprise, the mass spectrometry revealed no evidence of the presence of C_{21} steroids but, rather, indicated the presence of a $C_{19}O_3$ steroid, which was ultimately identified as 16β-hydroxydehydroepiandrosterone (16β-OH-DHEA).

To the best of our knowledge, 16β-OH-DHEA had never previously been assayed for mineralocorticoid activity. Therefore, we obtained some synthetic steroid from Professor W. Klyne, Curator of the Medical Research Council Steroid Reference Collection in London, and assayed it in our adrenalectomized rats. In repeated assays 16β-OH-DHEA had about 1/40th the potency of aldosterone; its potency as a mineralocorticoid was approximately that of 11-deoxycorticosterone.

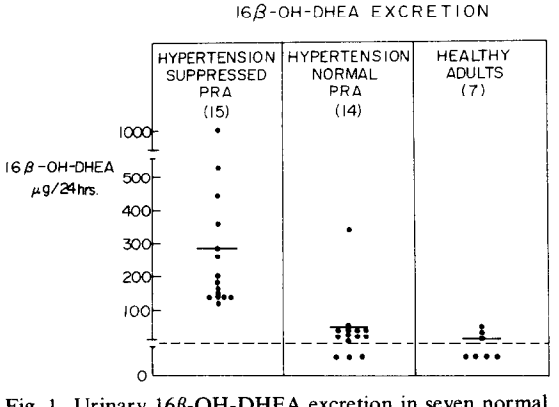

Fig. 1. Urinary 16β-OH-DHEA excretion in seven normal adults, 15 patients with low renin essential hypertension, and 14 patients with hypertension and non-suppressed renin (three of the last group had renovascular hypertension).

In a further attempt to verify the fact that 16β-OH-DHEA was a mineralocorticoid, we demonstrated that we could block its effect on sodium and potassium excretion in the adrenalectomized rat by simultaneous administration of the mineralocorticoid antagonist, spironolactone. In further studies of this type, we also demonstrated that spironolactone blocked the mineralocorticoid activity of extracts of urine from patients with low-renin essential hypertension.

Finally, we have developed a rather elaborate method for assaying 16β-OH-DHEA in urine, using gas chromatography as the final step in purifying and quantifying the compound. In applying this method to urine of various groups of human subjects, we have found that patients with low-renin essential hypertension excrete substantially more 16β-OH-DHEA than do normotensive adults, patients with normal-renin essential hypertension or patients with renovascular hypertension (Fig. 1).

In the course of our studies, we observed that 16β-OH-DHEA was rather easily converted to its 16-oxo-17β-ol isomer (Fig. 2). When injected into adrenalectomized rats, this latter compound has approximately the same mineralocorticoid activity as 16β-OH-DHEA. Gas chromatographic analyses have indicated that the 16-oxo-17β-ol isomer is also present in greater abundance in the urine of patients with low-renin essential hypertension than in other groups of subjects. Since both isomers are found in increased quantities and since both appear to have mineralocorticoid activity, it is possible that both are of importance in the pathogenesis of hypertension. Alternatively, it is possible that only one isomer is truly active and that the other must be isomerized in order to be biologically active.

It is obvious that a vast amount of work must be done before we shall have an adequate understanding of the role of 16β-OH-DHEA and its 16-oxo-17β-ol isomer in health and disease. It is not known whether these steroids are secreted by the adrenal cortex or are converted by another organ from some precursor such as DHEA. It is not known what regulates the production of these steroids, what their con-

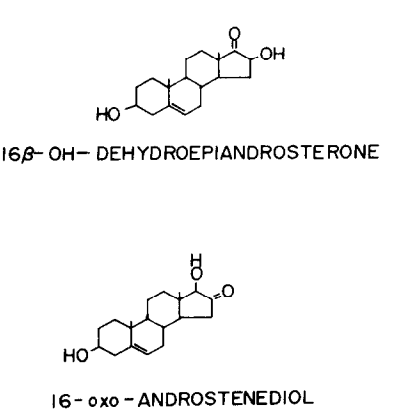

Fig. 2. Structure of 16β-OH-DHEA and its 16-oxo-17β-ol isomer.

centrations in the circulation might be, or what their production rates might be in various groups of subjects. It is not known how long these steroids might be elevated prior to the appearance of hypertension.

At present, all we can say with certainty is that circumstantial evidence led us to search for an unidentified mineralocorticoid in urine of patients with low-renin essential hypertension and that the search culminated in the discovery of two "new" steroids with mineralocorticoid activity... 16β-hydroxydehydroepiandrosterone and its 16-oxo-17β-ol isomer.

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