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19-Noraldosterone

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CONTENTS

I. INTRODUCTION

The 19-normineralocorticoids often possess higher mineralocorticoid and hypertensinogenic activities than their 19-substituted mother compounds.[1,2] Patients with primary aldosteronism have blood pressure and electrolyte abnormalities out of proportion to the degree of aldosterone secretion.[3,4] In these patients, abnormalities of the 19-nor-deoxycorticosterone (19-nor-DOC) have been reported.[5] The 19-noraldosterone, which was recently synthesized, possesses potent mineralocorticoid and hypertensinogenic activity.[6–8] 18,19-Dihydroxycorticosterone [18,19-$(OH)_2$-B] and 18-hydroxy-19-norcorticosterone (18-OH-19-nor-B), a possible precursor of 19-noraldosterone, have been identified in human urine.[9,10] They possess a hypertensinogenic activity.[11,12] The recently synthesized 18-deoxy-19-noraldosterone has been shown to be a potent aldosterone antagonist *in vitro*.[13,14] Figure 1 shows the structural formula of these mineralocorticoid hormones.

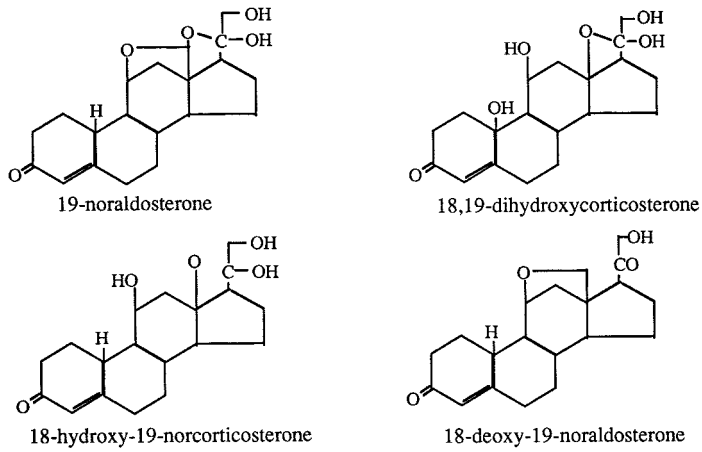

Figure 1 Structural formula of 19-noraldosterone, 18,19-dihydroxycorticosterone, 18-hydroxy-19-norcorticosterone, and 18-deoxy-19-noraldosterone.

II. BIOSYNTHESIS AND METABOLISM

A. BIOSYNTHESIS

Aldosterone is synthesized in the cells of the zona glomerulosa, the outermost of the three layers forming the cortex of the adrenal gland. Localization of aldosterone synthase, cytochrome P450aldo or C18 is strictly confined to the outermost cell layers in the zona glomerulosa.[15] A biosynthetic pathway leading to 19-nor-DOC has been proposed as follows: DOC → 19-OH-DOC → 19-oxo-DOC → 19-COOH-DOC → 19-nor-DOC. The last step is believed to take place extraadrenally, most likely in the kidney.[16] However, Azar and Melby reported higher concentration of plasma 19-nor-DOC in the adrenal vein of aldosterone-producing adenoma.[17] 19-Nor-DOC may be synthesized partly in the aldosterone-producing adenoma. 19-Noraldosterone is identified in the incubation medium of aldosterone-producing adenoma and normal adrenal tissue using HPLC-MS (Figure 2). In addition to 19-noraldosterone, 18,19-$(OH)_2$-B and 18-OH-19-nor-B are found in the incubation medium of adrenal tissues using specific radioimmunoassays (RIAs) performed after a sequence of HPLC systems.[18] The rates of production of the three steroids are high in the aldosterone-producing adenomas and in adrenal hyperplasia compared with those in either Cushing's adenoma or "silent" adenoma (Figure 3).

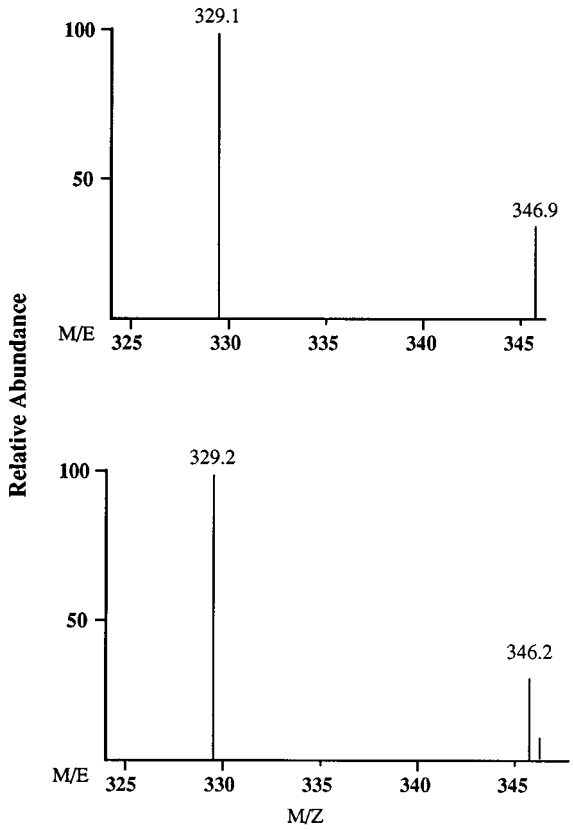

Figure 2 Mass spectra of synthetic 19-noraldosterone (upper) and the sample of 19-noraldosterone isolated from the adrenal incubation medium (lower).

B. BIOSYNTHESIS AND METABOLISM OF 19-NORALDOSTERONE IN THE RAT

The biosynthetic pathway of 19-noraldosterone is reported in isolated rat glomerulosa cells (GC) and fasciculata-reticular cells (FC) by analyzing [^{14}C]-pregnenolone metabolism using HPLC and quantification by specific RIA. In GCs, 18,19-$(OH)_2$-B is detected after 15 min incubation with [^{14}C]-pregnenolone, 18-OH-19-nor-B is detected 30 min after incubation, and 19-noraldosterone is detected after 45 min incubation before aldosterone reaching the peak (Figure 4). These three mineralocorticoids are not detected in FCs.[19] These results demonstrate that 19-noraldosterone is synthesized in the glomerulosa cells and then may undergo further metabolism.

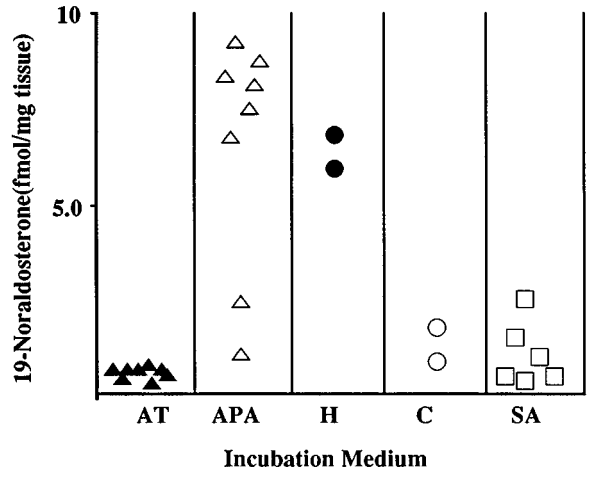

Figure 3 19-Noraldosterone in the incubation medium of adjacent adrenal tissue (AT), aldosterone-producing adenoma (APA), adrenal hyperplasia (H), adenoma of Cushing's syndrome (C), and "silent" adenoma (SA).

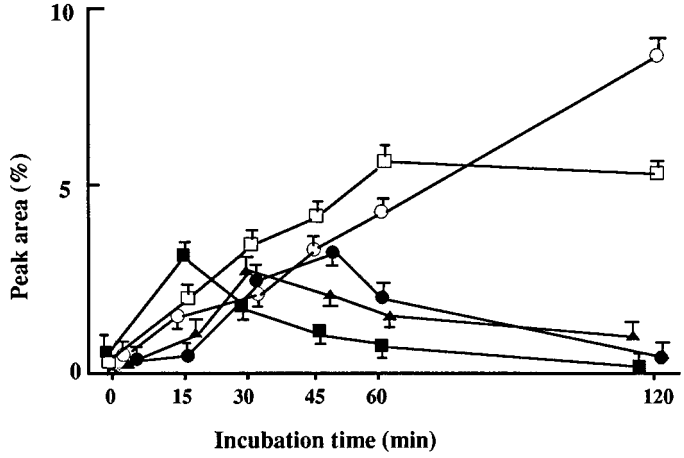

Figure 4 Time course for the formation and subsequent metabolism of 19-noraldosterone by adrenal zona glomerulosa cells. Adrenal zona glomerulosa cells were incubated with 20 n*M* of [^{14}C]-pregnenolone. (●) 19-noraldosterone, (■) 18,19-dihydroxycorticosterone, (▲) 18-hydroxy-19-norcorticosterone, (□) 18-hydroxycorticosterone, (○) aldosterone.

C. BIOSYNTHESIS OF 18-DEOXY-19-NORALDOSTERONE

18-Deoxy-19-noraldosterone, which has been synthesized, is a potent aldosterone antagonist.[13,14] This steroid hormone is identified by mass spectrometry from human aldosterone-producing adenoma and adrenal tissue incubated with synthetic 19-noraldosterone (Figure 5). 18-Deoxy-19-noraldosterone-like immunoreactivity is detected in the aldosterone-producing adenoma fraction incubated without synthetic 19-noraldosterone.[20] These results suggest that 18-deoxy-19-noraldosterone is synthesized in the adrenal gland. A few cases of primary aldosteronism without hypertension have been reported.[21,22] However, the reason for the absence of hypertension in these cases with primary aldosteronism has not been elucidated yet. Normokalemic primary aldosteronism has been reported;[23,24] however, the definitive factors causing normokalemia remain to be established. It is interesting to see whether or not 18-deoxy-19-noraldosterone is involved as an antagonist in any of the above-mentioned forms of primary aldosteronism.

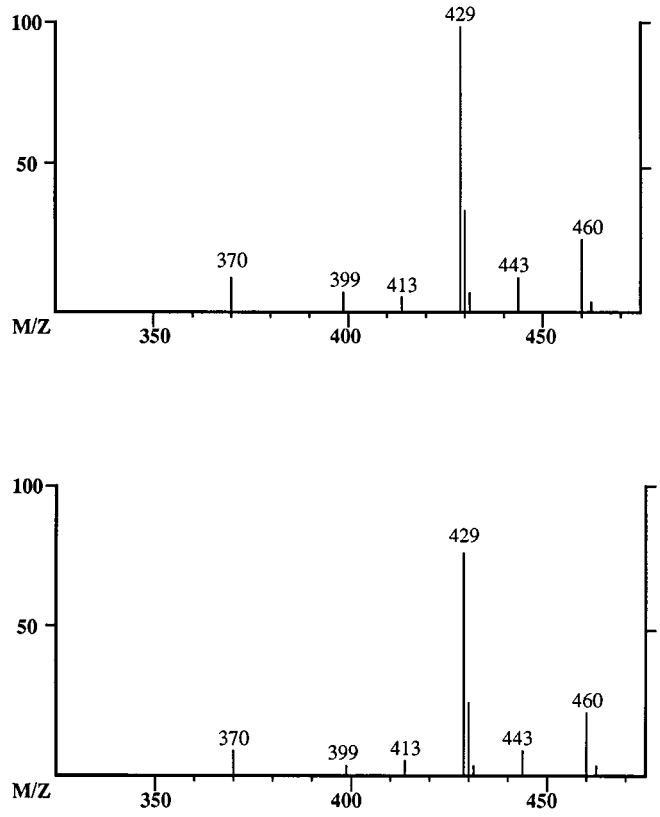

Figure 5 Mass spectra of synthetic 18-deoxy-19-noraldosterone (top) and 18-deoxy-19-noraldosterone isolated from incubated aldosterone-producing adenoma (bottom).

III. METHODS OF DETERMINATION OF 19-NORALDOSTERONE IN URINE

Urinary free 19-noraldosterone, free 18,19-$(OH)_2$-B and free 18-OH-19-nor-B are measured by RIA after the purification of urine extracts by HPLC. Ten to 50 ml of urine containing [^{3}H]-labeled each steroid (3000 cpm) was extracted with Sep-pak C18 cartridge (Waters, Milford, MA) and chromatographed in a reversed-phase HPLC system, followed by RIA using a specific antibody and individual recovery measurements. A C-18 column was used with a solvent system: water/acetonitrile/methanol (72:23:5, v/v/v) a flow rate of 2 ml/min. Retention times of 19-noraldosterone, 18,19-$(OH)_2$-B, 18-OH-19-nor-B, and aldosterone were 24, 14, 18, and 32 min, respectively. Aliquots of the respective fractions were evaporated under a stream of compressed air, and the steroid content was measured using the specific RIA. Table 1 shows the cross-reactivity between the antibody of each steroid and various steroid compounds. The sensitivity of each assay was 30 fmol per tube. The overall recovery was 60% to 70% for 19-noraldosterone, 50% to 60% for 18,19-$(OH)_2$-B, and 40% to 50% for 18-OH-19-nor-B. The interassay variations were 13.5% for 19-noraldosterone, 14.5% for 18,19-$(OH)_2$-B, and 14.9% for 18-OH-19-nor-B. The intraassay variations for 19-noraldosterone, 18,19-(OH)2-B and 18-OH-19-nor-B were 9.2%, 9.5%, and 10.5%, respectively.

IV. CONTROL OF 19-NORALDOSTERONE SECRETION

A. RENIN-ANGIOTENSIN SYSTEM

19-Noraldosterone secretion is controlled by the renin-angiotensin system. After sodium restriction, the 24-h urinary excretion of 19-noraldosterone increases (Figure 6), which is parallel with aldosterone excretion.[25] The concentration of 19-noraldosterone increases in the medium of human cultured adrenal zona glomerulosa cells incubated with angiotensin II. Angiotensin II may directly stimulate 19-noral-dosterone secretion in the adrenal zona glomerulosa cells.

Table 1 Cross-Reactivity Between the Antibody (Ab) of 18,19-Dihydroxycorticosterone, 18-Hydroxy-19-Norcorticosterone and 19-Noraldosterone and Various Steroid Compounds

	Ab: 18,19-$(OH)_2$-B	18-OH-19-nor-B (% cross-reaction)	19-noraldo
18,19-$(OH)_2$-B	100	0.1	<0.02
18-OH-19-nor-B	3.2	100	<0.02
18-OH-B	1.5	3.8	0.08
Aldo	<0.02	<0.02	193
19-noraldo	<0.02	0.5	100
18-OH-A	1.5	6.7	0.46
18-OH-DOC	0.6	1.2	19
Cortisol (F)	<0.02	<0.02	<0.02
Cortisone	<0.02	<0.02	<0.02
6-OH-F	<0.02	<0.02	<0.02
18-OH-progesterone	<0.02	<0.02	0.2
Corticosterone	<0.02	<0.02	0.29
19-OH-aldo	<0.02	<0.02	13
Tetrahydroaldo	<0.02	<0.02	0.87
17-OH-progesterone	<0.02	<0.02	<0.02
18-OH-F	<0.02	<0.02	<0.02

Note: A, dehydrocorticosterone DOC, deoxycorticosterone 18,19-$(OH)_2$-B, 18,19-dihydroxycorticosterone 18-OH-19-nor-B, 18-hydroxy-19-nor-corticosterone 19-noraldo, 19-noraldosterone 18-OH-F, 18-hydroxycortisol.

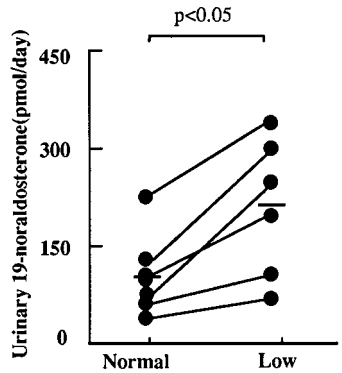

Figure 6 Effects of sodium restriction on urinary excretion of 19-noraldosterone in six normal subjects. Sodium restriction was achieved by 4 days of a low sodium diet (50 mmol/day) after a normal sodium-containing diet (200 mmol/day). The excretion of 19-noraldosterone increased significantly after the low sodium diet. Horizontal lines indicate the mean ± SEM.

Aldosterone secretion is controlled by the renin-angiotensin system and potassium. Recently aldosterone synthase (cytochrome P450 aldo or C18) was identified; cDNA clones encoding the enzyme have been isolated.[26,27] Acute and chronic stimuli, such as sodium restriction, potassium and angiotensin II loading, have been reported to increase the enzyme activity and messenger RNA of aldosterone synthase but do not increase cytochrome P450 11ß, cortisol synthase.[28,29] Little is known about the enzyme synthesizing of 19-noraldosterone. Further studies are necessary to clarify the closer pathways of the production of this steroid, including the synthetic enzyme.

B. ADRENOCORTICOTROPIC HORMONE

The concentration of 19-noraldosterone increases in the medium of human cultured adrenal zona glomerulosa cells incubated with adrenocorticotropic hormone (ACTH). ACTH is one of the stimulators of 19-noraldosterone from the adrenal zona glomerulosa cells.

Acute ACTH administration increases aldosterone release,[30] whereas continuous ACTH administration results in a transient increase in plasma aldosterone and urinary aldosterone, which reach a peak after 48 h and return to baseline values by 72–96 h.[31,32] Both ACTH and angiotensin II increase the activity and expression of mRNA of cytochrome P450 aldo or C18.[33,34] The explanation for the transient increase in aldosterone secretion despite continuous ACTH administration is not clear.

The 19-nor-DOC is relatively unresponsive to acute changes in dietary sodium intake.[35] The precursors of 19-nor-DOC are under the regulation of ACTH in the fasciculata of the adrenal cortex.[16] ACTH increases the concentration of the circulating precursors and may increase 19-nor-DOC.

C. AGING

Aldosterone secretion declines with advancing age.[36] Plasma renin activity (PRA) is also decreased in aged subjects.[37] The effect of aging on the urinary excretion of 19-noraldosterone and 18,19-$(OH)_2$-B in normal subjects was investigated. There was a negative correlation between age and 19-noraldosterone (Figure 7). Urinary 18,19-$(OH)_2$-B decreased with aging. Urinary free cortisol did not correlate with age.[38] This decrease in PRA with increasing age is most likely the primary cause of the lower aldosterone and 19-noraldosterone values in older subjects. Little is known about the cause of the decline in PRA and adrenal sensitivity to angiotensin II with aging.

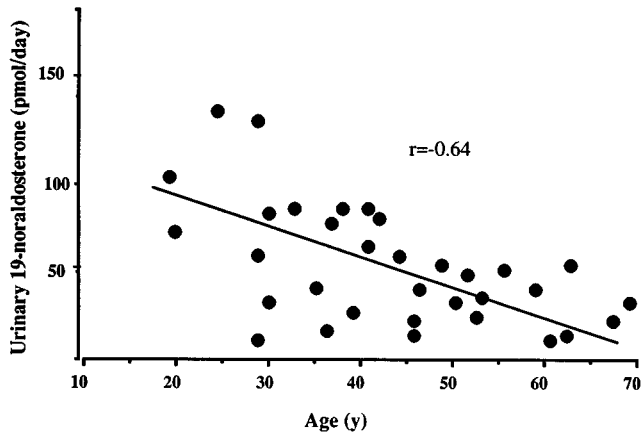

Figure 7 Correlation between age and urinary excretion of 19-noraldosterone in normal subjects. There was a significant negative correlation between age and 19-noraldosterone.

V. 19-NORALDOSTERONE IN PRIMARY ALDOSTERONISM

Patients with primary aldosteronism frequently have the abnormalities of blood pressure and electrolyte disproportionate to the degree of aldosterone secretion.[3,4] In these patients, abnormalities of the potent mineralocorticoid active steroid 19-nor-DOC have been reported.[5] Urinary excretions of 19-noraldosterone, 18,19-$(OH)_2$-B, and 18-OH-19-nor-B are elevated in patients with primary aldosteronism or secondary aldosteronism.[10] Figure 8 shows the urinary excretion of 19-noraldosterone in patients with primary aldosteronism, secondary aldosteronism, essential hypertension , or normal controls. Urinary 18-hydroxycortisol (18-OH-F) levels are useful markers for differential diagnoses of the sub-types of primary aldosteronism.[39] The usefulness of measuring the levels of urinary 19-noraldosterone, 18,19-$(OH)_2$-B and 18-OH-19-nor-B was examined to distinguish patients with aldosterone-producing adenoma (APA) from those with idiopathic hyperaldosteronism (IHA), and compared with two diseases with respect to the urinary excretion of aldosterone, 18-OH-B and 18-OH-F. Urinary excretions of 19-noraldosterone, 18,19-$(OH)_2$-B, 18-OH-19-nor-B, 18-OH-B, 18--OH-F, and aldosterone were measured in 25 patients with primary aldosteronism, 16 with APA, and 9 with IHA. High concentrations of plasma aldosterone in both adrenal veins and the absence of a solitary adenoma on CT scan of the adrenal glands confirmed the diagnosis of IHA.

Other biological parameters, including low serum potassium concentrations, suppressed PRA, and high plasma aldosterone concentrations, were similar in both groups. The urinary excretion of aldosterone, 18,19-$(OH)_2$-B and 18-OH-19-nor-B did not differ in patients with APA from in those with IHA.

In patients with IHA the urinary 19-noraldosterone level was lower than in patients with APA; however, the difference did not reach the significant level (Figure 9). The urinary excretion of 18-OH-B and 18-OH-F in patients with APA were significantly higher than in patients with IHA (Figure 9). Urinary 18-OH-F and 18-OH-B may be a useful marker to diagnose and distinguish the two subsets of primary aldosteronism. Urinary excretion of 19-noraldosterone may be an indicator for primary aldosteronism, but it does not differentiate between the two subtypes of aldosteronism. Aldosterone and 18-OH-F are both synthesized by the same enzyme, aldosterone synthase (P450 aldo or C18).[27] The expression of mRNA of P450 aldo was increased in aldosteronoma.[40] The discrepancy between aldosterone and 18-OH-F in idiopathic hyperaldosteronism should be further clarified.

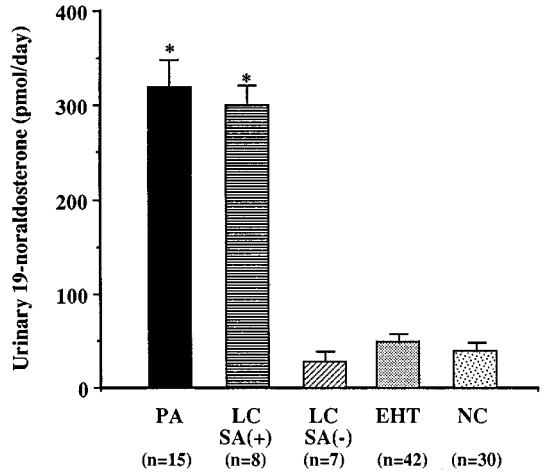

Figure 8 Comparison of urinary 19-noraldosterone in patients with primary aldosteronism (PA), liver cirrhosis with (LC, SA(+)) and without (LC, SA(-)) aldosteronism, and essential hypertension (EHT), and in normal subjects (NC). *P <.05 vs. LC, SA(-), EHT, and NC.

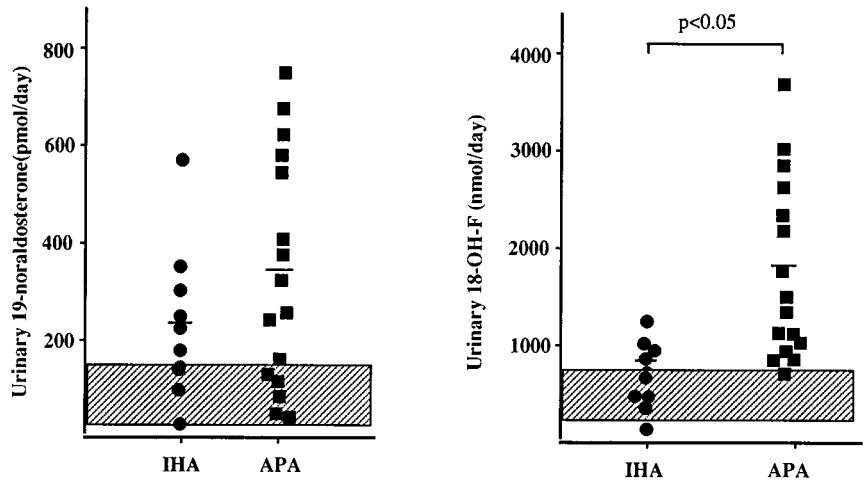

Figure 9 Urinary excretion of 19-noraldosterone and 18-hydroxycortisol in patients with aldosterone-producing adenoma (APA) (n = 16) or idiopathic hyperaldosteronism (IHA) (n = 9). There was no significant difference in 19-noraldosterone levels between patients with APA and those with IHA. Urinary 18-OH-F levels were significantly greater in patients with APA than those with IHA (P <.05).

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