19-Noraldosterone, a New Mineralocorticoid

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Introduction

The 19-nor steroid derivatives often possess higher mineralocorticoid and hypertensinogenic activities than their 19-substituted mother compounds [1, 2]. In particular, extensive biochemical and clinical studies have been performed on 19-nordeoxycorticosterone (19-nor-DOC). The 19-noraldosterone, which was recently synthesized [3] possesses potent mineralocorticoid and hypertensinogenic activity [4, 5]. 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 [6, 7]. They possess a hypertensinogenic activity [8, 9]. The recently synthesized 18-deoxy-19-noraldosterone has been shown to be a potent aldosterone antagonist *in vitro* [10, 11]. Fig. 1 shows the structural formula of these mineralocorticoid hormones. The proposed biosynthetic pathway of 19-noraldosterone was shown in Fig. 2.

Biosynthesis of 19-noraldosterone

19-Nor-DOC has been proposed to be synthesized extraadrenally, most likely in the kidney [12]. However, Azar and Melby reported higher concentration of plasma 19-nor-DOC in the adrenal vein of aldosterone-producing adenoma [13]. We isolated and identified 19-noraldosterone in the incubation medium of aldosterone-producing

adenoma and adrenal tissue using HPLC-MS (Fig. 3). In addition to 19-noraldosterone, 18, 19-(OH)₂-B and 18-OH-19-nor-B were found in the incubation medium of adrenal tissues using specific RIAs performed after a sequence of HPLC systems. The rates of production of the three steroids were high in the aldosterone-producing adenomas and in adrenal hyperplasia compared with in either Cushing's adenoma or "silent" adenoma (Fig. 4).

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 has been isolated [14, 15]. 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 not increase cytochrome P450_{11β}, cortisol synthase [16–18]. To elucidate the regulatory mechanism for 19-noraldosterone, we studied the effect of sodium restriction on the urinary excretion of 19-noraldosterone and 18, 19-(OH)₂-B in six normal subjects. 19-Noraldosterone, 18, 19-(OH)₂-B, 18-hydroxycorticosterone (18-OH-B), and 18-hydroxycortisol (18-OH-F) were measured by specific RIAs after purification of the urine extract by HPLC [7, 19–21]. Table 1 summarizes the results of the responses of these steroids to sodium restriction. The 24-h urinary excretion of 19-noraldosterone and 18, 19-(OH)₂-B were increased approximately 2-fold ($P < 0.05$) by sodium restriction. Virtually identical responses were seen for aldosterone, 18-OH-B, and 18-OH-F. These observations suggest that 19-noraldosterone and 18, 19-(OH)₂-B are partly under the control of the renin-angiotensin system in normal subjects.

Aldosterone secretion declines with advancing age. Plasma renin activity (PRA) is also decreased in aged subjects. We investigated the effect of aging on the urinary excretion of 19-noraldosterone

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

Fig. 2. The proposed biosynthetic pathway of 19-noraldosterone.

and 18, 19-(OH)₂-B in normal subjects. Significant negative correlation between age and 19-noraldosterone was observed ($r = -0.64$, $P < 0.05$) (Fig. 5). Urinary 18, 19-(OH)₂-B, aldosterone, 18-OH-B and 18-OH-F decreased with aging ($r = -0.65$, -0.78 , -0.66 , -0.49 , $P < 0.05$, respectively). Urinary free cortisol did not correlate with age. These results suggest that angiotensin may contribute to chronic regulation of 19-noraldosterone and 18, 19-(OH)₂-B. The lack of relationship between cortisol and 19-noraldosterone suggests that ACTH does not always regulate 19-noraldosterone. The 19-nor-DOC is relatively unresponsive to acute changes in dietary sodium intake [22]. The precur-

Fig. 3. Mass spectra of synthetic 19-noraldosterone (upper) and the sample of 19-noraldosterone isolated from the adrenal incubation medium (lower).

Table 1. Plasma renin activity, urinary excretion of 19-noraldosterone, 18,19-(OH)₂-B and 18-OH-F in response to sodium intake

Sodium intake	PRA (ng/L.s)	19-Noraldo (pmol/day)	18,19-(OH) ₂ -B (nmol/day)	18-OH-F (nmol/day)
Normal (180 mmol/day)	0.20 ± 0.06	107 ± 40	5.6 ± 0.8	377 ± 93
Low (50 mmol/day)	0.89 ± 0.28*	259 ± 76*	15.6 ± 4.5*	554 ± 129*

Values are shown as the mean ± SEM for six normal subjects. 19-Noraldo, 19-noraldosterone; 18,19-(OH)₂-B, 18,19-dihydroxycorticosterone; 18-OH-F, 18-hydroxycortisol. *: $P < 0.05$ vs. normal sodium intake.

Fig. 4. 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). **Fig. 5.** Correlation between age and urinary excretion of 19-noraldosterone in normal subjects. There was a significant negative correlation between age and 19-noraldosterone.

sors of 19-nor-DOC are under the regulation of ACTH in the fasciculata of the adrenal cortex [12]. ACTH increases the concentration of the circulating precursors and may increase 19-nor-DOC. 18-OH-F is regulated by both ACTH and renin-angiotensin [20, 23]. The enzyme cytochrome P450_{aldo or C18} synthesizes 18-OH-F from cortisol [15]. Decrease of urinary 18-OH-F with aging suggests that 18-OH-F is chronically regulated with angiotensin.

Aldosterone is synthesized in the glomerulosa cells and aldosterone synthase, P450_{aldo or C18} exists only in the adrenal zona glomerulosa [24]. We investigated the biosynthetic pathway of 19-noraldosterone in isolated rat glomerulosa cells (GC) and fasciculata-reticular cells (FC) by analyzing [¹⁴C]-pregnenolone metabolism using HPLC and quantification by specific RIA. In GCs, 18, 19-(OH)₂-B was detected after 15 min incubation with [¹⁴C]-pregnenolone, 18-OH-19-nor-B was detected after

30 min after incubation, and 19-noraldosterone was detected after 45 min incubation, before the appearance of an aldosterone peak (Fig. 6). These three mineralocorticoids were not detected in FCs. We previously found that 19-noraldosterone was converted to 18-deoxy-19-noraldosterone in human adrenal gland [25]. These results demonstrate that 19-noraldosterone is synthesized in the glomerulosa cells and then may undergo further metabolism.

19-Noraldosterone in primary aldosteronism

Patients with primary aldosteronism frequently have blood pressure and electrolyte abnormalities disproportionate to the degree of aldosterone secretion [26, 27]. In these patients, abnormalities of the potent mineralocorticoid active steroid 19-

nor-DOC has been reported [28]. We found elevated urinary excretion of 19-noraldosterone, 18, 19-(OH)₂-B, and 18-OH-19-nor-B in patients with primary aldosteronism or secondary aldosteronism [7]. Fig. 7 shows the urinary excretion of 19-noraldosterone in patients with primary aldosteronism, secondary aldosteronism, essential hypertension, or normal controls. We reported that urinary 18-OH-F levels were useful markers in differentially diagnosing the sub-types of primary aldosteronism [29]. We examined the usefulness of measuring the levels of urinary 19-noraldosterone, 18, 19-(OH)₂-B and 18-OH-19-nor-B 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 excretion 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,

Fig. 6. Time course for the formation and subsequent metabolism of 19-noraldosterone by adrenal zona glomerulosa cells. Adrenal zona glomerulosa cells were incubated with 20 nM of [¹⁴C]pregnenolone. (●) 19-noraldosterone, (■) 18,19-dihydroxycorticosterone, (▲) 18-hydroxy-19-norcorticosterone, (□) 18-hydroxycorticosterone, (○) aldosterone.

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, but this difference was not significant (Fig. 8). The urinary excretion of 18-OH-B and 18-OH-F in patients with APA were significantly higher than in patients with IHA (Fig. 8). Urinary 18-OH-F and 18-OH-B may be useful marker to diagnose and distinguish the two subsets of primary aldosteronism. However, urinary 19-noraldosterone could be used to diagnose primary aldosteronism but not distinguish the two sub-types of aldosteronism.

Aldosterone and 18-OH-F are both synthesized by the same enzyme, aldosterone synthase (P450_{aldo} or C18) [15]. The expression of mRNA of P450_{aldo} was increased in aldosteronoma [30]. The discrepancy of aldosterone and 18-OH-F in idiopathic hyperaldosteronism should be further clarified.

18-Deoxy-19-noraldosterone which is recently synthesized is a potent aldosterone antagonist [11]. We identified 18-deoxy-19-noraldosterone by mass spectrometry from human aldosterone-producing adenoma and adrenal tissue incubated with synthetic 19-noraldosterone (Fig. 9). A few cases of primary aldosteronism without hypertension have

Fig. 7. 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*<0.05 vs. LC, SA(-), EHT, and NC.

Fig. 8. Urinary excretion of 19-noraldosterone and 18-hydroxycortisol (18-OH-F) 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 IHA. Urinary 18-OH-F levels were significantly greater in patients with APA than IHA ($P < 0.05$).

Fig. 9. Mass spectra of synthetic 18-deoxy-19-noraldosterone (top) and 18-deoxy-19-noraldosterone isolated from incubated aldosterone-producing adenoma (bottom).

been reported [31, 32]. However, the reason for the absence of hypertension in these cases of primary aldosteronism has not been elucidated yet. Normokalemic primary aldosteronism has been reported [33, 34], but the definitive factors causing normokalemia remain to be established. It will be 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.

In conclusion, the 19-noraldosterone possesses a potent mineralocorticoid and hypertensinogenic activity. 19-Noraldosterone was synthesized in adrenal glands and regulated by renin-angiotensin system. The production of 19-noraldosterone increased in aldosterone-producing adenoma and urinary excretion was elevated in patients with primary hyperaldosteronism. However, nothing is known about the enzyme of synthesis of 19-noraldosterone. Further studies are necessary to clarify the closer pathway(s) of the production of this steroid including the synthetic enzyme. Additionally, the clinical significance of 19-noraldosterone should be studied in large clinical samples.

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