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Role of 21-Deoxyaldosterone in Human Hypertension

Saleh Abdelhamid,[1]* Sabina Lewicka,[2] Katharina Bige,[2] Doris Haack,[2] Horst Lorenz,[3] Uschi Nensel,[1] Arnold Röckel,[1] Peter Fiegel,[1] Dieter Walb[1] and Paul Vecsei[2]

[1] *Hypertension and Nephrology Unit, Deutsche Klinik für Diagnostik, Wiesbaden,* [2] *Department of Pharmacology, University of Heidelberg and* [3] *Institute for Biometry and Statistics, Neuberg, Germany*

21-Deoxyaldosterone has been postulated to be a precursor of aldosterone in an altenative biosynthesis pathway and Kelly's-M1 is considered to be its metabolite. In healthy volunteers, the excretion rate of 21-deoxyaldosterone and of Kelly's-M1 are significantly lower than the aldosterone metabolites, aldosterone-18-glucuronide and tetrahydro-aldosterone and than the aldosterone precursor 18-OH-corticosterone. Essential hypertension patients (with low and normal renin) excrete comparable values of 21-deoxyaldosterone and Kelly's-M1 as normotensives. In 66% of aldosterone-producing adenoma cases (APA) and in 60% of idiopathic hyperaldosteronism (IHA) patients, significantly raised values of 21-deoxyaldosterone and Kelly's-M1 were found. The patients with the high excretion rates of both steroids showed only moderately increased values of the aldosterone metabolites, aldosterone-18-glucuronide and tetrahydro-aldosterone, as well as of the aldosterone precursor 18-OH-corticosterone. In contrast, the latter mentioned steroids were excreted in higher amounts in those patients with normal excretion of 21-deoxyaldosterone and Kelly's-M1. Hence, it is suggested that aldosterone is produced alternatively either via 18-OH-corticosterone alone or additionally via 21-deoxyaldosterone. Furthermore, in three cases of "incidentally" discovered adrenal adenomas, 21-deoxyaldosterone and Kelly's-M1 were the only elevated steroids. After adrenalectomy, excretion of 21-deoxyaldosterone and of Kelly's-M1 and blood pressure returned to normal, which proves that these steroids play a role in blood pressure regulation. In essential hypertension, ACTH infusion induced a significant increase of 21-deoxyaldosterone and Kelly's-M1. However, the increase after angiotensin II was 3- to 6-fold higher than after ACTH. IHA patients proved to be more responsive to angiotensin II; and, in contrast, APA cases proved to be more sensitive to ACTH. The data suggest that beside the main route of aldosterone biosynthesis via 11-deoxycorticosterone, corticosterone and 18-OH-corticosterone an alternative pathway exits via 21-deoxyaldosterone in healthy and in hypertensive patients. There are similarities between the regulation of 21-deoxyaldosterone and the regulation of aldosteorne. The determination of 21-deoxyaldosterone and its possible metabolite Kelly's-M1 might be appropriate in the diagnosis of mineralocorticoid-induced forms of hypertension, especially when an adrenal adenoma is discovered.

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INTRODUCTION

Aldosterone is normally produced from cholesterol via pregnenolone, progesterone, 11-deoxycorticosterone (11-DOC), corticosterone and probably 18-OH-corticosterone (main route of aldosterone synthesis [1–3]. An alternative pathway via 21-deoxyaldosterone (21-D-Ald) was first described by Wettstein [4], and demonstrated in bovine adrenals by Kahnt and Neher [5], in humans and bullfrogs by Ulick [3] and in adrenal tissue of rats by Lewicka *et al.* [6]. The last group also showed that 21-D-Ald is mainly formed in the zona glomerulosa. Determination of 21-D-Ald is therefore likely to be important in the diagnosis of adrenal dysfunction.

*Correspondence to S. Abdelhamid.
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An earlier study characterized the specificity of a tetrahydro-aldosterone (3α,5β-) radioimmunoassay; less polar material with tetrahydro-aldosterone-like immunoactivity was found in large amounts in urine samples of patients with low renin hypertension and primary aldosteronism [7]. Fisher *et al.* [8] found this material or a part of it to be identical with the substance 11β:18 (s), 18:20α-diepoxy-5β-pregnan-3α ol. This substance was first described and designated M1 by Kelly *et al.* [9]. It was found in the urine of subjects given huge amounts of aldosterone. Lewicka *et al.* [10], developed a radioimmunoassay which could show that it is a natural product. They provided evidence that it is a breakdown product of 21-D-Ald.

The aim of the present study was to determine the clinical significance of these two steroids (21-D-Ald and Kelly's-M1) as compared to the metabolites of aldosterone and the aldosterone precursor 18-OH-corticosterone and to examine the influence of the physiological regulatory mechanisms on their excretion rates. Urinary 21-D-Ald and Kelly's-M1 and also aldosterone-18-glucuronide, tetrahydro-aldosterone and 18-OH-corticosterone were determined in normal subjects, in patients with essential hypertension and in patients with different forms of primary aldosteronism. These metabolites were also determined in three cases with "incidentally" discovered adrenal adenomas. Furthermore, it is known that many factors—notably ACTH, sodium and, more importantly, angiotensin II—determine the rate of aldosterone secretion. The response of aldosterone to these stimuli differs in patients with hypertension, with aldosterone-producing adenomas and with idiopathic hyperaldosteronism (associated with bilateral hyperplasia of the cortex). There is so far no data on the response of 21-D-Ald and Kelly's-M1 to these stimuli in humans. Therefore the effects of ACTH, angiotensin II, diuretic treatment and saline infusions on 21-D-Ald and Kelly's-M1 were also studied in some patients with these forms of hypertension.

SUBJECTS AND METHODS

Clinical studies

Subjects and patients. Normal subjects and five groups of patients with different forms of hypertension were studied as shown in Table 1. The normotensive healthy volunteers had no history of high blood pressure. Hypertensive cases were referred by general practitioners or by other hospitals for further investigations. All patients had been off antihypertensive medication and spironolactone for at least 2 and 6 weeks, respectively. The diagnosis of essential hypertension (EH) was established after all possible causes of secondary hypertension had been ruled out. Low renin essential hypertension was defined as a plasma renin activity of <0.45 ng angiotensin I/ml·h in persons standing upright. Patients with essential hypertension and elevated plasma renin were excluded from the study. Patients with primary aldosteronism were identified by low plasma renin activity, elevated urinary aldosterone metabolites and elevated plasma aldosterone values. Aldosterone-producing adenomas (APA) and idiopathic hyperaldosteronism (IHA) differentiation was based on the results of at least three of the following procedures: postural aldosterone response, plasma and urinary 18-OH-corticosterone determination, computed tomography of the adrenals, and dexamethasone-suppressed adrenal scintigraphy. The diagnosis of APA was proved by surgery in all cases. The large number of IHA cases in this study, which are even more numerous than the APA cases, is at variance with literature data; this is because more adenomas are being diagnosed and operated on in other hospitals due to the widely used CT technique. Furthermore, one third of our cases with hyperplasia were already known to the unit and were recalled for participation.

Three cases whose history of hypertension was not known before this investigation were monitored in addition because of adrenal adenomas discovered fortuitously by CT. In the course of the investigation, the following values were obtained: blood pressure

Table 1. Number, gender and age of the population studied

Groups	Males			Females			Total Number of cases
	n	Age in years median	Range	*n*	Age in years median	Range	
Normal subjects	13	29	(22–52)	18	36	(23–69)	31
Low renin essential hypertension	10	47	(35–66)	12	49	(33–66)	22
Normal renin essential hypertension	45	46	(16–68)	24	46	(17–66)	69
Aldosterone-producing adenomas (APA)	18	46	(30–58)	17	46	(23–64)	35
Idiopathic hyperaldosteronism (IHA)	30	52	(24–66)	19	43	(22–66)	49
"Incidentaloma"	1		48	2		43/50	3

170–180/95–105 mmHg, sodium 142–145 and potassium 3.9–4.0 mequiv/l; renin lying 0.1–0.3 and standing 0.1–0.8 ng angiotensin I/ml·h; free cortisol, deoxycorticosterone and catecholamines were normal.

Steroid studies

Determination of basic values of the urinary steroids. While consuming their usual diet, subjects and patients collected 24 h urine samples which were kept refrigerated until analysis. In all urines, Kelly's-M1 and 21-D-Ald, aldosterone-18-glucuronide (Ald-18-G), tetrahydro-aldosterone (TH-Ald) and 18-OH-corticosterone (18-OH-B) were determined.

Stimulation and suppression tests. These were performed only on patients who gave written informed consent after detailed explanation. The study was approved by the ethics commission of the clinic.

ACTH (25 IU Synacthen, Ciba). ACTH in 500 ml 5% laevulose solution was infused over a period of 4 h in 15 cases with EH (7 with low renin and 8 with normal renin), 15 cases with APA and 11 with IHA. 24 h urine specimens were collected the day prior to and the day of the test.

Angiotensin II (Hypertensin-Ciba). Angiontensin II in 5% laevulose solution was administered at rates of 0.1, 0.5, 1.0, 2.0 and 4.0 ng/kg/min (23–25) to 7 patients with EH (3 with low and 4 with normal renin), 5 with APA and 4 with IHA. Each rate was continued for 1 h except the last step; this was adjusted to the blood pressure reactions. An exaggerated rise of blood pressure was therefore not observed. Urine was collected on the day prior to and on the day of the test.

Diuretic treatment. Twenty cases of EH (10 with low and 10 with normal renin) were treated with a combination of 5 mg amiloride–HCl and 50 mg hydrochlorothiazide daily. 24 h urine samples were obtained before and on the 8th–10th day of medication.

Saline infusion. Two litres of 0.9% sodium chloride were given over a period of 4 h to 37 patients with EH (19 with low renin and 18 with normal renin), 22 with APA and 36 with IHA. 24 h urine was sampled the day prior to and on the day of examination.

Analytical methods

The urinary steroids Kelly's-M1, 21-D-Ald, Ald-18-G, TH-Ald and 18-OH-B were determined by radioimmunoassay after extraction and repeated chromatographic purification as reported previously [10–15]. In the case of Kelly's-M1 and TH-Ald, the urine samples were first treated with glucuronidase. 21-D-Ald and Ald-18-G were measured after 24 h acid hydrolysis (pH 1). Urine samples were not pretreated for 18-OH-B analysis.

Statistical analysis

Basic statistics were computed for measured variables (median, minimum, maximum, 25–75% quantiles). For reasons of robustness, nonparametric data analysis was done. To describe correlations among the variables, Spearman's rank correlation coefficients were calculated. To compare test-induced changes of the measured steroids, a percentage change was calculated according to the formula:

$$\text{Percent change} = (\text{Post-test value} - \text{Pre-test value})/\text{Pre-test value} \times 100.$$

Wilcoxon's rank test (for matched pairs) was used to establish significance. The level of significance was set at 5%.

RESULTS

Results of basic values of the urinary steroids in normal subjects and in hypertensive patients

Normal values. The excretion rates of normal subjects are depicted in Table 2. Kelly's-M1 and 21-D-Ald were excreted in significantly ($P < 0.01$) lower excretion rates than Ald-18-G, TH-Ald and 18-OH-B.

EH patients. EH patients excreted comparable amounts of the steroids (Table 2) to those of normotensive subjects. There were no significant differences between low and normal renin EH subgroups. Females and males excreted comparable amounts of the steroids. Age-related differences in the excretions of Kelly's-M1 and 21-D-Ald reached significance only in the normotensive subjects. In normal subjects < 50 years ($n = 25$) the median of Kelly's-M1 was 2.1 and 21-D-Ald was 0.9 μg/24 h. In subjects > 50 years ($n = 6$), the median of Kelly's-M1 was 1.1 and of 21-D-Ald was 0.4 μg/24 h. The differences for Ald-18-G, TH-Ald and 18-OH-B between the age groups mentioned were small; however, there were too few individuals aged $\geqslant 60$ years for a meaningful evaluation of this age group. Whilst lower values for Kelly's-M1 and 21-D-Ald were already seen after th age of 50, Ald-18-G, TH-Ald and 18-OH-B showed lower values only after the age of 60 years [16–18].

Patients with primary aldosteronism. In accordance with the results of Kelly's-M1 and 21-D-Ald both APA and IHA patients were divided into two discrete subgroups (Table 2):

(i) In a first subgroup of 23 of the 35 APA cases (65.7%) and 29 of the 49 IHA cases (59.2%), Kelly's-M1 and 21-D-Ald were excreted in significantly larger amounts than in normal individuals and EH patients.

(ii) In a second subgroup of 12 out of the 35 APA cases (34.3%) and 20 of the 49 IHA cases (40.8%) Kelly's-M1 and 21-D-Ald were repeatedly in the normal range. Of note is that the values of Ald-18-G, TH-Ald and 18-OH-B were significantly higher in this second subgroup than in the first, showing an inverse relation between these steroids and Kelly's-M1 and 21-D-Ald.

The differences between these subgroups were neither sex- nor age-related.

Table 2. Urinary excretion rates of Kelly's-M1 (KM1), 21-deoxyaldosterone (21-D-Ald), aldosterone-18-glucuronide (Ald-18-G), tetrahydroaldosterone (TH-Ald) and 18-OH-corticosterone as well as K-M1/21-D-Ald ratio in normal subjects, essential hypertensive patients and cases with primary aldosteronism

Patient diagnosis	μg/24 h					
	KM1	21-D-Ald	KM1/ 21-D-Ald	Ald-18-G	TH-Ald	18-OH-B
Normal subjects $n = 31$						
Range	0.1–3.0	0.1–2.9	0.1–5.0	3.5–17.5	10.0–70.0	1.5–6.5
Median	1.9	0.7	2.4	6.1	33.0	3.1
25–75% quant.	1.0–2.5	0.5–1.0	2.0–3.0	4.5–9.6	21.8–40.3	2.6–4.2
Low renin essential hypertension: $n = 22$						
Range	0.3–3.0	0.3–2.9	0.2–3.7	3.5–16.5	13.4–70.0	1.5–6.2
Median	1.5	0.9	1.7	7.1	40.9	3.8
25–75% quant.	1.0–2.0	0.9–1.0	1.0–3.0	5.0–10.0	33.0–52.0	3.0–5.0
Normal renin essential hypertension: $n = 69$						
Range	0.1–3.0	0.2–3.0	0.1–6.0	3.5–15.9	10.5–69.7	1.5–6.3
Median	1.7	0.8	2.0	7.9	43.9	4.1
25–75% quant.	1.0–2.0	0.4–1.3	1.0–3.0	5.0–11.0	32.0–55.0	3.0–5.0
Aldosterone-producing adenoma (APA): 1st subgroup $n = 23$ patients						
Range	1.1–70.5	1.3–40.3	0.2–2.8	7.3–72.5	64.9–536.8	5.1–33.1
Median	7.3	5.7	1.5	20.0	100.2	9.4
25–75% quant.	4.4–17.0	3.0–10.0	1.0–2.0	15.0–29.0	86.0–135.0	8.0–14.0
Aldosterone-producing adenoma (APA): 2nd subgroup $n = 12$ patients						
Range	1.0–3.0	0.5–3.0	0.3–4.0	13.4–42.5	102.9–544.0	9.3–36.1
Median	2.1	1.7	1.2	23.9	148.9	14.8
25–75% quant.	2.0–3.0	1.0–2.0	1.0–2.0	20.0–35.0	121.0–176.0	13.0–19.0
Idiopathic hyperaldosteronism (IHA): 1st subgroup $n = 29$ patients						
Range	1.1–16.7	0.4–10.3	0.2–5.0	10.0–58.9	40.8–480.5	1.6–7.6
Median	5.5	3.9	1.6	18.4	96.3	4.7
25–75% quant.	4.0–8.0	2.0–5.0	1.0–2.0	16.0–23.0	82.0–132.0	4.0–6.0
Idiopathic hyperaldosteronism (IHA): 2nd subgroup $n = 20$ patients						
Range	0.9–3.0	0.3–3.0	0.4–3.3	8.6–51.4	48.8–481.0	3.4–8.9
Median	2.3	1.4	1.5	23.5	127.4	5.5
25–75% quant.	2.0–3.0	1.0–2.0	1.0–2.0	17.0–26.0	90.0–155.0	5.0–6.0

Cases with APA and cases with IHA were divided into two subgroups. In the first subgroup, KM1 and/or 21-D-Ald were elevated. In the second subgroup, they were consistently within the normal range (in 3 controls).

In the population studied, the correlation between Kelly's-M1 and 21-D-Ald in normotensive subjects was $r = 0.61$ ($P < 0.05$); in EH $r = 0.53$; in patients with APA $r = 0.94$; and with IHA $r = 0.61$. There were no correlations either between Kelly's-M1 or between 21-D-Ald and Ald-18-G, TH-Ald and 18-OH-B in any individual.

Three cases with "incidentally" discovered adrenal adenomas. Kelly's-M1 and 21-D-Ald were the only elevated urinary steroids as shown in Fig. 1. Free DOC and 18-OH-DOC were also normal. After operation, Kelly's-M1 and 21-D-Ald normalized, the other steroids remained normal, in patients No. 2 TH-Ald excretion decreased by 50%, sodium was 140–144, and potassium 4.0–4.5 mequiv/l; lying renin was 0.3–1.2 and standing renin 0.7–1.8 ng angiotensin I/ml·h. Blood pressure was normalized in two and improved in one patient during an observation period of 2 to 3 years.

Results of stimulation and suppression tests

Effect of ACTH administration (Fig. 2). ACTH significantly increased the excretion of Kelly's-M1 and 21-D-Ald in EH patients (both with low and normal renin) and, even more markedly, in APA patients. The response achieved in cases with IHA was lower than in APA patients. Both subgroups (as defined in Table 2) of APA and of IHA patients showed comparable percentage change of Kelly's-M1 and of 21-D-Ald before and after ACTH (Fig. 2) but at different levels. The values in the first subgroup were higher than in the second subgroup. The Kelly's-M1/21-D-Ald ratios before and after the test were almost similar.

Results of angiotensin II infusion (Fig. 2). Angiotensin II greatly increased the excretion of Kelly's-M1 and of 21-D-Ald in essential hypertension (with comparable % changes in low and normal renin subgroups); this increase was 3- to 6-fold higher than the response

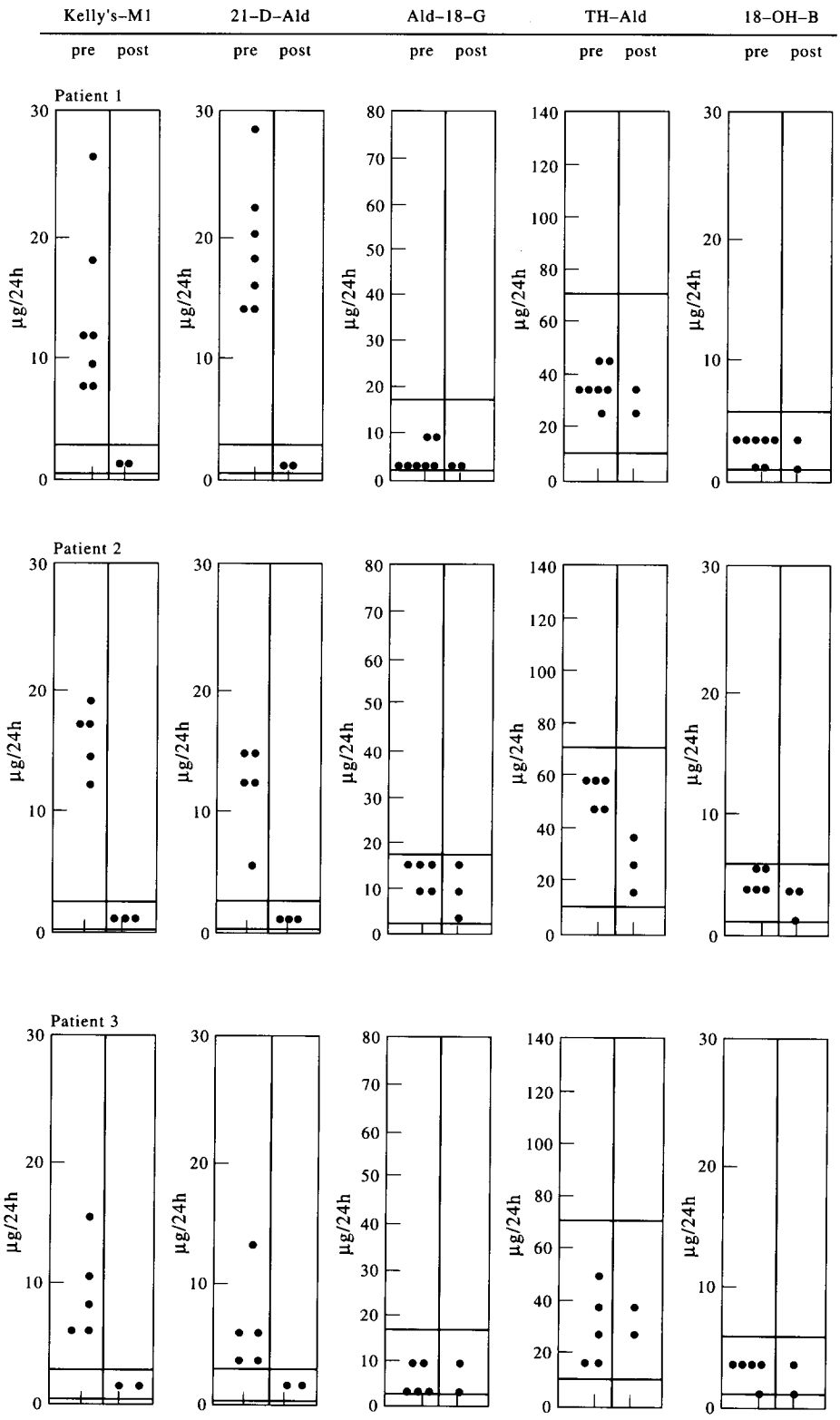

Fig. 1. Urinary excretion rates (pre- and post-operation values) of Kelly's-M1, 21-deoxyaldosterone (21-D-Ald), aldosterone-18-glucuronide (Ald-18-G), tetrahydro-aldosterone (TH-Ald) and 18-OH-corticosterone (18-OH-B) in 3 cases with "incidentally" discovered adrenal adenomas.

obtained after ACTH administration. Similar results as in EH cases were seen in the IHA patients. The values were significantly lower in APA patients. Both subgroups first and second of the APA and of IHA patients (defined in Table 2) showed comparable changes after angiotensin II administration; however, this was at different levels. The values in the first subgroup were higher than in the second subgroup.

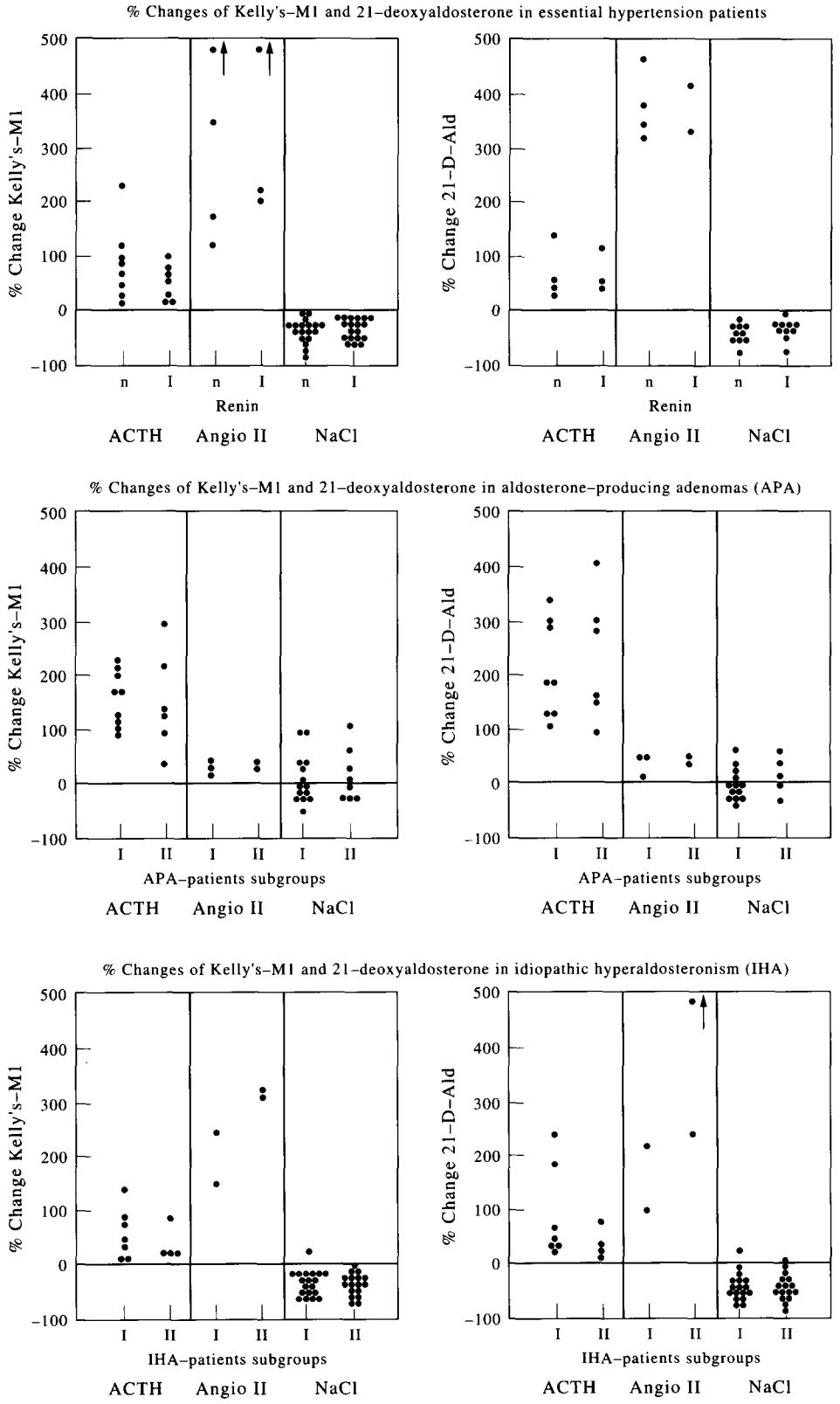

Fig. 2. Individual responses of the urinary Kelly's-M1 and 21-deoxyaldosterone (as percent change of the post-test from pre-test values) to ACTH, angiotensin II and 2 l 0.9% saline infusion in patients with essential hypertensin (n = normal, l = low renin), APA and IHA (both first and second subgroups as in Table 2).

Results of diuretic treatment (Table 3). In the treated patients, Kelly's-M1 and 21-D-Ald increased significantly without relevant differences between low and normal renin subgroups. The percentage changes were near to those obtained after angiotensin II in the EH cases. The Kelly's-M1/21-D-Ald ratios remained almost unchanged before (2.03) and after (2.0) diuretic treatment.

Table 3. Changes of Kelly's-M1 and 21-D-Ald excretion by treatment with diuretics (5 mg amiloride and 50 mg hydrochlorothiazide) in 20 cases with essential hypertension (pre- and post-treatment values and calculated % changes)

	μg/24 h					
	Kelly's-M1 diuretic			21-deoxyaldosterone diuretic		
	Pre	Post	%-change	Pre	Post	%-change
Low renin essential hypertension: $n = 10$						
Median	1.6	5.8	284.1	1.0	3.1	230.6
Range	0.8–2.4	4.0–11.5	115.8–737.5	0.4–2.6	1.2–6.8	82.4–733.3
Normal renin essential hypertension: $n = 10$						
Median	1.6	4.6	185.7	1.0	2.9	190.0
Range	0.3–7.2	0.8–10.6	52.8–404.8	0.4–2.3	1.2–7.2	62.5–407.7

Effect of saline infusion (Fig. 2). Saline infusion induced a significant reduction of Kelly's-M1 and 21-D-Ald in both EH and IHA patients. A relevant reduction was observed in only 31% of APA cases. The percentage changes manifested in the subgroups of both APA and IHA patients after treatment were almost similar to those before treatment, but this was at different levels. The values in the first subgroup were higher than in the second one.

DISCUSSION

Normal subjects and patients with EH (with no significant differences between low and normal renin) excrete comparable amounts of 21-D-Ald and Kelly's-M1. The production of 21-D-Ald and Kelly's-M1 is significantly lower than that of the aldosterone and the aldosterone precursor 18-OH-B, indicating that the main route of the aldosterone synthesis is the more active one, but that the alternative pathway is also operative.

In primary aldosteronism, significantly raised excretion rates of Kelly's-M1 and 21-D-Ald were found in 65.7% of APA and in 59.2% of IHA cases. The remainder of the patients with normal values of these two steroids did show higher values of Ald-21-G, TH-Ald, and especially of 18-OH-B than the first group. This indicates that aldosterone is produced alternatively either via 18-OH-B alone or additionally via 21-D-Ald in hyperaldosteronism. Since patients with APA and IHA can excrete normal values of the aldosterone metabolites Ald-18-G or TH-Ald [17, 18], the determination of Kelly's-M1 and/or 21-D-Ald can be of additional use as a diagnostic marker for patients with hypertension and/or suspected adrenal disorders.

The significance of 21-D-Ald and of Kelly's-M1 is further shown in the three cases in this study with "incidentally" discovered adrenal adenomas. They excreted large amounts of Kelly's-M1 and 21-D-Ald, while the excretions of Ald-18-G, TH-Ald, 18-OH-B and DOC were repeatedly normal. After surgery, excretions of the elevated steroids normalized and over an observation period of 2 to 3 years blood pressure became normal in two patients and improved in one. This shows that beside the "classic" Conn's syndrome there is a subset of adrenal adenomas in which the alternative aldosterone pathway is hyperactive with hyper-21-deoxy-aldosteronism, that can be diagnosed only by determination of 21-D-Ald and Kelly's-M1. It is known however that intestinal bacteria can metabolize aldosterone to 21-D-Ald during its enterohepatic circulation [19, 20], this cannot be the cause of the high amounts of 21-D-Ald in our cases.

21-D-Ald has mineralocorticoid activity almost equivalent to that of 11-DOC and binding to the mineralocorticoid receptor similar to that of aldosterone, as shown by Koshida *et al.* [21]. These properties and the normalization of blood pressure in our cases with incidentally discovered adenomas suggest that 21-D-Ald may have hypertensinogenic activity either by itself or by amplifying the effects of aldosterone.

Lewicka *et al.* [6] had investigated some mechanisms possibly involved in the regulation of 21-D-Ald in adrenal tissues of rats. It is known that the renin–angiotensin system dominates the control of aldosterone secretion and that ACTH is a short-term stimulator of aldosterone formation by acting on both the early and late steps of the biosynthesis [1, 22–25]. In our cases with EH, ACTH administration enhanced the secretion of 21-D-Ald (and consequently the production of Kelly's-M1) as it did for Ald-18-G and TH-Ald as well as for 18-OH-B. However, the stimulation induced by angiotensin II infusion was three to six times higher than after ACTH. Angiotensin II provoked a high increase of 21-D-Ald and of Kelly's-M1, comparable to that of Ald-18-G and TH-Ald, and to that of 18-OH-B. Furthermore, the activation of the endogenous renin–angiotensin system induced by diuretic treatment in 20 EH cases resulted in a significant increase of 21-D-Ald and Kelly's-M1, similar to that occurring after angiotensin II infusion. In contrast, sodium loading and volume expansion by saline infusions (which result in a fall of angiotensin II) caused an inhibition of 21-D-Ald and of Kelly's-M1, as did Ald-18-G [26], TH-Ald and 18-OH-B. The results of these stimulation and suppression tests show that the

production of 21-D-Ald (and Kelly's-M1) is regulated in a similar manner to aldosterone.

Aldosterone secretion under different test conditions differs between patients with primary aldosteronism due to adrenal adenomas (APA) and patients with primary aldosteronism due to bilateral adrenal hyperplasia (IHA). APA are more sensitive to ACTH and show little dependence on the renin–angiotensin system, whereas the adrenals are hypersensitive to angiotensin II stimulation in IHA [22–26]. When our patients from both APA and IHA subgroups were examined they showed similar percentage changes of 21-D-Ald and Kelly's-M1 under test conditions, but this was at different levels. The ACTH-induced increases of 21-D-Ald and of Kelly's-M1 in APA cases were four to five times higher than in IHA patients. In contrast, and as expected, the response in IHA patients of 21-D-Ald and Kelly's-M1 to angiotensin II was three to seven times higher than in APA cases. This was reflected by the suppression of angiotensin II which caused a significant inhibition of 21-D-Ald and Kelly's-M1 in 91% of the patients with IHA, but only in about 31% of the APA cases. These results again show similarities between the responses of aldosterone and 21-D-Ald under test conditions in APA and IHA cases, which can be of use in the differential diagnosis of these disorders.

Concerning the relation between 21-D-Ald and Kelly's-M1 there is evidence that the second is mainly the breakdown product of the first. This is supported by the following observations. There is a correlation between these two compounds in the individuals studied. The ratio Kelly's-M1/21-D-Ald remained similar before and after the administration of ACTH and angiotensin II, as well as after saline infusions and diuretic treatment. Furthermore, Lewicka *et al.* [10, 11] showed high values of 21-D-Ald and Kelly's-M1 in the 21-hydroxylase-deficient form of congenital adrenal hyperplasia with low aldosterone production. However, a comparison of Kelly's-M1 and 21-D-Ald values in our study—shown by the median in Table 2—revealed higher values of the first compound than of the second in many cases. This can be attributed to the fact that 21-D-Ald occurs in the urine in different forms as shown by Lewicka *et al.* [11, 27]. In parallel to the aldosterone catabolism, 21-D-Ald may be conjugated in part in the kidney in the same manner as aldosterone and can be directly excreted as 21-D-Ald-18-G. Most of the 21-D-Ald may be metabolized in the liver to the tetrahydro-compound and either excreted as 21-deoxy-TH-Ald or further metabolized to Kelly's-M1. High values of 21-deoxy-TH-Ald and Kelly's-M1 could be found in cases with 21-hydroxylase deficiency [10, 11, 27]. Kelly's-M1 may therefore be the metabolite of 21-D-Ald-18-G as well as of 21-deoxy-TH-Ald. In the present study, 21-D-Ald-18-G and Kelly's-M1, but not 21-deoxy-TH-Ald were determined. A dissociation may occur in the relation between 21-D-Ald-18-G and 21-deoxy-TH-Ald—as known from the metabolism of aldosterone, where a dissociation can occur between Ald-18-G and TH-Ald [17, 18]. In some of our patients, the metabolism of 21-D-Ald could have occurred mainly via 21-deoxy-TH-Ald and Kelly's-M1. For this reason Kelly's-M1 can be higher than 21-D-Ald-18-G in such cases. Therefore, determination of two of the three metabolites (21-D-Ald-18-G and/or Kelly's-M1 and/or 21-deoxy-TH-Ald) is necessary to detect cases with suspicion of high 21-D-Ald production.

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