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Physiologic roles of 11β-hydroxysteroid dehydrogenase type 2 in kidney

Yukinori Isomura[a], Tomoatsu Mune[a,*], Hiroyuki Morita[b], Tetsuya Suwa[a], Nobuki Takada[c], Yoritsuna Yamamoto[c], Jun Takeda[a]

[a]*Department of Diabetes and Endocrinology, Gifu University School of Medicine, Gifu 501-1194, Japan*
[b]*Department of General Medicine, Gifu University School of Medicine, Gifu 501-1194, Japan*
[c]*Japan Self-defense Force Gifu Hospital, Kakamigahara, Gifu 504-8701, Japan*

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Abstract

We developed enzyme-linked immunosorbent assays to measure urinary free cortisone (E) and cortisol (F) and analyzed correlations between clinical measures reflecting mineralocorticoid action and 24-hour urinary excretion of E and F or their ratio, uE/F, which has been considered as the most sensitive index of renal 11β-hydroxysteroid dehydrogenase type 2 activity. Two hundred nineteen healthy men were enrolled in this study. The uE/F ratio was 1.10 ± 0.41 (mean ± SD), and a strong linear correlation between uE and uF was observed in a double reciprocal plot. Urinary acid-labile aldosterone excretion had a negative correlation with 24-hour urinary Na excretion and Na/K ratio, but uE/F ratio had a weak positive correlation with the Na/K ratio and no significant correlation with 24-hour urinary Na excretion. In contrast, uE and uF had positive correlations with 24-hour urinary excretions of Na and K, raising the possibility of separate renal effects mediated by the glucocorticoid receptor. Furthermore, uE and uE/F ratio had strong negative correlations with urinary concentrations of Na and K. These results suggest that renal 11β-hydroxysteroid dehydrogenase type 2 is an important regulatory factor of renal Na and K handlings independently of and/or complementary to the mineralocorticoid action of aldosterone.

1. Introduction

11β-Hydroxysteroid dehydrogenases (11βHSDs) are microsomal enzymes responsible for the interconversion between active 11β-hydroxyglucocorticoids and their receptor inactive 11-ketometabolites, cortisol (F) and cortisone (E), respectively, in humans [1,2]. Two cloned isozymes accounting for 11βHSD activity [3,4], type 1 (HSD11B1) and type 2 (HSD11B2), have different characteristics in enzyme kinetics, molecular biology, and physiologic roles.

HSD11B1 is a nicotinamide adenine dinucleotide phosphate (oxidized [$NADP^+$] or reduced [NADPH] form)–dependent enzyme with a high K_m for F or E in the micromolar range, which is above the physiologic range of circulating free F (~10 nmol/L). HSD11B1 is expressed most abundantly in the liver and colocalized with glucocorticoid receptor. Its activity is bidirectional in tissue homogenates or isolated microsomal fractions, but the isozyme acts predominantly as an 11β-reductase in primary culture of hepatocytes or dispersed cells. Thus, HSD11B1 regenerates active glucocorticoids from their inactive 11-ketometabolites [5,6].

In contrast, HSD11B2 is a NAD^+-dependent 11β-dehydrogenase with a low K_m in the nanomolar range, which is close to the physiologic concentration of plasma free F, and colocalized with mineralocorticoid receptor. In vitro, F and aldosterone have similar affinity for the mineralocorticoid receptor. Plasma F, mostly protein bound, circulates at 100 to 1000 times higher levels than aldosterone, and levels of plasma free F are still likely higher. Selectivity and specificity of aldosterone action within target organs are mediated by HSD11B2, by converting F to E, whereas aldosterone is not metabolized [7,8]. Defects in HSD11B2 activity, either congenital in the syndrome of apparent mineralocorticoid excess or acquired after licorice ingestion, result in F-induced mineralocorticoid hypertension [9,10].

To assess the HSD11B2 activity in vivo, 24-hour urinary ratio of (tetrahydrocortisol [THF] + alloTHF]/tetrahydrocortisone (THE) measured by gas chromatography/mass spectrometry has been used as an index. However, the ring A–reduced metabolites of F and E are partially influenced

* Corresponding author. Tel.: +81 58 230 6568; fax: +81 58 230 6568.
E-mail address: mune@cc.gifu-u.ac.jp (T. Mune).

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by the HSD11B1, and the complexity of the method for measurement restricts the clinical use. Recent studies revealed that the ratio of urinary free F to E reflects renal HSD11B2 activity more accurately than the urinary ratio of (THF + alloTHF) to THE [11-16]. In addition, kidney HSD11B2 activity is believed to regulate the mineralocorticoid receptor action, but the fundamental relationship between the HSD11B2 activity and renal electrolyte handling has not been assessed. We have developed enzyme-linked immunosorbent assays (ELISAs) to measure urinary free E and F, and analyzed associations of 24-hour urinary E (uE), F (uF), or the ratio of E/F (uE/F) with clinical measures reflecting mineralocorticoid action in healthy men.

2. Subjects and methods

2.1. Subjects

The study population was randomly selected from subjects visiting for medical checkups at the Japan Self-defense Force Gifu Hospital; subjects gave written informed consent. Subjects were kept on a normal diet and under air-conditioned circumstances during hospitalization. The ethical committee of Gifu University School of Medicine approved the study protocol. Body height and weight were recorded with subjects wearing light clothing, and body surface area was calculated with the following formula: body surface area (m^2) = (body weight)$^{0.425}$ (kg) × (body height)$^{0.725}$ (cm) × 0.007184. Blood pressure (BP) at rest was measured with a mercury sphygmomanometer with the subject in a sitting position, and the mean of 3 readings taken 5 minutes apart was used. A total of 219 healthy male subjects not taking any medications were enrolled. Their mean age and body mass index (±SD) were 48 ± 6 years (range, 28-59) and 23.6 ± 2.5 kg/m^2 (range, 17.1-32.3), respectively. Mean systolic and diastolic BP were 124 ± 17 and 73 ± 10 mm Hg, respectively.

2.2. Measurement of electrolyte, creatinine, and aldosterone levels

Twenty-four-hour urine was collected in a plastic container without preservatives during hospitalization and an aliquot stored at −20°C. Electrolyte and creatinine levels in urine and sera were measured by ion-selective electrode and Jaffe method, respectively. The 24-hour urinary acid–labile aldosterone excretion (uAld) was measured as follows: an aliquot of urine was acidified to pH 1.0 with 1 N HCl for hydrolysis, kept for 24 hours, extracted, and uAld was measured by using an Aldosterone-RIA Kit II (Dinabot RI Laboratory, Tokyo, Japan). Means of duplicate or triplicate determinations were used in all calculations.

2.3. Reagents for ELISAs

F, E, F-peroxidase, bovine serum albumin (BSA), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and thimerosal were obtained from Sigma Chemical (St Louis, MO). Goat anti–rabbit IgG Fc antibody, horseradish peroxidase avidin D, and urea peroxide were purchased from Rockland (Gilbertsville, PA), Vector Laboratories (Burlingame, CA), and Calbiochem (La Jolla, CA), respectively. [1,2,6,7-^{3}H]F (specific activity, 74 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). [1,2,6,7-^{3}H]E was produced by incubating [1,2,6,7-^{3}H]F with renal microsomes from an adrenalectomized rat for 60 minutes followed by extraction and purification with thin-layer chromatography (Whatman, Clifton, NJ) developed with 25% acetone in methylene chloride. Ninety-six-well plates (Nunc-Immuno Plate Maxisorp Surface) for ELISA were obtained from Nalge Nunc International (Rochester, NY). E-3-carboxymethoxylamine (CMO)-biotinamidecaproylhydrazide (E-biotin) was prepared as described previously [17]. Anti-F or anti-E antiserum was developed by immunization of F-3-CMO-BSA or E-3-CMO-BSA with Freund adjuvant to New Zealand white rabbits. Cross-reactivities of the antibodies were assessed by incubation with increasing concentrations of competing steroids. These steroids were obtained from Sigma Chemical and Steraloids (Newport, RI). The concentration that produced an optical density 50% of the zero in the standard curve was used for calculation of the relative cross-reactivity. The results are listed in Table 1.

2.4. Measurement of urinary F and E concentrations

We measured 24-hour uF and uE concentrations by using the respective ELISAs. One hundred microliters of urine and 100 μL of phosphate-buffered saline (PBS) (20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4, with 0.01% thimerosal) containing 4000 dpm of [1,2,6,7-^{3}H]F was extracted with 4 mL of methylene chloride by shaking for

Table 1
Cross-reactivities of cortisone and cortisol antibodies

Steroid	E antibody (%)	F antibody (%)
Cortisol	3.4	100.0
Cortisone	100.0	4.2
11-Dehydrocorticosterone	0.6	<0.1
11-Deoxycortisol	0.6	2.0
Corticosterone	<0.1	0.3
Deoxycorticosterone	<0.1	<0.1
Progesterone	<0.1	<0.1
6β-Hydroxycortisol	<0.1	0.4
18-Hydroxycortisol	<0.1	<0.1
20β-Dihydrocortisol	0.1	2.6
20α-Dihydrocortisone	0.2	<0.1
20β-Dihydrocortisone	<0.1	<0.1
α-Cortol	<0.1	<0.1
α-Cortolone	<0.1	<0.1
5α-THF	<0.1	7.5
THF	<0.1	0.3
THE	0.2	0.1
11β-Hydroxyetiocholanolone	<0.1	<0.1
Aldosterone	<0.1	<0.1
Cortol	<0.1	<0.1
Cortolone	<0.1	<0.1

Table 2
Urinary hormones and their correlations with electrolytes

	uE		uF		uE/F ratio		uAld	
24-h Excretion, mean ± SD (median) (μg/d)	42.7 ± 19.8 (39.5)		45.1 ± 29.9 (35.0)				6.4 ± 4.1 (5.6)	
Parameter	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Serum Na (mEq/L)	−0.186	.0060	−0.187	.0060	0.020	NS	0.092	NS
Serum K (mEq/L)	−0.253	.0002	−0.240	.0004	0.052	NS	0.134	.0470
Urinary Na [mEq (g-Cre)$^{-1}$ m^{-2} d^{-1}]	0.477	<.0001	0.336	<.0001	0.124	NS	−0.211	.0020
Urinary K [mEq (g-Cre)$^{-1}$ m^{-2} d^{-1}]	0.337	<.0001	0.294	<.0001	−0.007	NS	0.097	NS
Urinary Na/K ratio	0.290	<.0001	0.172	.0140	0.140	.0460	−0.353	<.0001
Ck (L/d)	0.354	<.0001	0.245	.0004	0.107	NS	−0.175	.0120
FENa (%)	0.499	<.0001	0.280	<.0001	0.212	.0023	−0.250	.0003
FEK (%)	0.453	<.0001	0.258	.0002	0.188	.0069	−0.168	.0160
Urinary Na (mEq/L)	−0.363	<.0001	0.004	NS	−0.453	<.0001	−0.073	NS
Urinary K (mEq/L)	−0.428	<.0001	−0.095	NS	−0.427	<.0001	0.220	.0012

Pearson's correlation analysis was used to analyze the relationships between urinary hormone excretions (in micrograms per gram of creatinine excretion per square meter of body surface area) and the above parameters after adjusting for age and diastolic BP. NS indicates nonsignificant; Ck, clearance; FENa, fractional excretions of Na; FEK, fractional excretions of K.

10 minutes. After centrifugation and aspiration of the aqueous layer, the organic layer was evaporated to dryness with a vacuum evaporator and reconstituted in 1 mL of PBS. Radioactivity of a 200-μL aliquot was measured in a liquid scintillation counter and used for correcting the recovery. Mean recovery rate for [1,2,6,7-^{3}H]F was 84.2% ± 11.2%. The mean recovery rate for [1,2,6,7-^{3}H]E (82.9% ± 5.1%) was almost the same as that for [1,2,6,7-^{3}H]F and the recovery of F was used to assess the recovery of both steroids.

For F, a 96-well ELISA plate was coated with 200 μL of rabbit anti-F polyclonal antibody diluted 1:3000 with 0.1 mol/L sodium carbonate buffer (pH 9.0) per well at 4°C overnight. The plates were washed 4 times with washing buffer (PBS with 0.1% Tween 20) by using an automatic microplate washer (Bio-Rad Laboratories, Hercules, CA). Then, triplicate 10-μL samples and triplicate 10-μL F standards containing 0, 10, 25, 50, 100, 250, 500, and 1000 pg were incubated with 0.2 μg/mL of F-labeled peroxidase in 200 μL of assay buffer (PBS with 0.5% BSA and 0.1% Tween 80, pH 7.4) per well at 4°C overnight. For E, an ELISA plate was coated overnight with 1 μg of goat anti–rabbit IgG Fc antibody in 200 μL of the carbonate buffer per well. Triplicate 10-μL samples and triplicate 10-μL E standards containing 0, 10, 25, 50, 100, 250, 500, and 1000 pg were incubated with biotin-conjugated E (1:5 000 000 dilution) and rabbit anti-E antiserum (1:10 000 dilution) in 200 μL of assay buffer per well at 4°C overnight. After the plate was washed, it was incubated with 1:300 diluted 0.5 mg/mL of horseradish peroxidase avidin D in 200 μL of assay buffer per well for 45 minutes at room temperature. For F and E, the washed plates were developed for 1 hour with 200 μL of 0.1 mol/L sodium citrate buffer (pH 4.0) containing 0.1 mg/mL urea peroxide and 0.4 mmol/L of ABTS and read at 405 nm with a microplate reader (Model 550, Bio-Rad Laboratories). The data were analyzed by using Microplate Manager software (Bio-Rad Laboratories). The lowest detection limit for both F and E was 200 pg/mL of urine. Means of triplicate determinations were used in all calculations. The intra- and interassay coefficients of variation were as follows: F, 6.9% and 14.9%, and E, 8.7% and 12.5%, respectively.

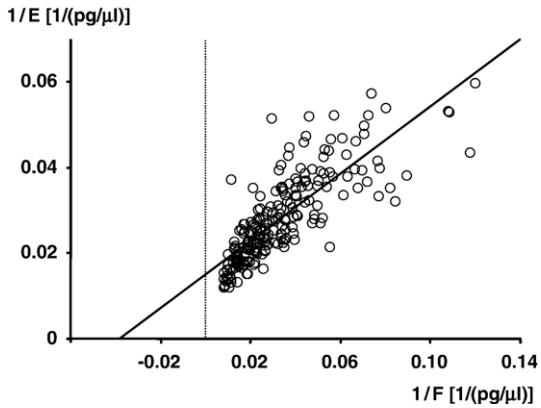

Fig. 1. A double reciprocal plot showing a strong linear correlation between uE and uF. The estimated K_m value for HSD11B2 clinically deduced from these individual measurements was 72 nmol/L, comparable to the values reported in vitro. Data were analyzed by using Pearson's correlation analysis.

2.5. Statistical analysis

Data are expressed as means ± SD. Statistical analyses were performed with SAS version 6.12 for Windows (SAS Institute, Cary, NC). Pearson's partial correlation analysis was used where appropriate. To correct variation in urine collection and a strong association with body stature, 24-hour urinary excretion of hormones or electrolytes were expressed as the quantity per gram of creatinine excretion (g-Cre) per square meter of body surface area.

3. Results

3.1. Twenty-four-hour urinary excretion of F, E, and Ald, and their relationships

uF, uE, and uAld excretions were 18.6 ± 11.1 (range, 4.3-70.2; median, 15.4), 18.1 ± 7.9 (range, 4.7-55.7; median, 16.5), and 2.6 ± 1.4 (range, 0.3-8.0; median, 2.4)

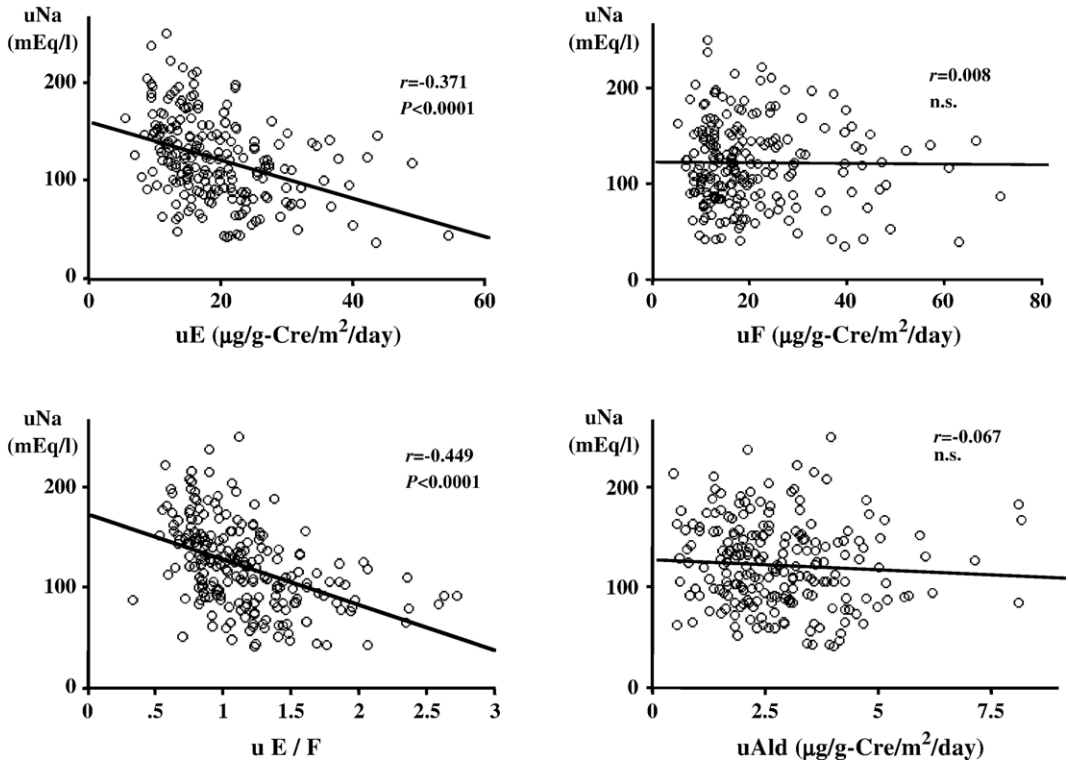

Fig. 2. Correlations between urinary concentration of sodium and uE, uF, uE/F, and uAld. The uE and uE/F had strong negative correlation with urinary concentration of sodium, whereas uF and uAld did not. Data were analyzed by using Pearson's correlation analysis.

μg (g-Cre)$^{-1}$ m^{-2}, respectively. The actual 24-hour excretion values are also shown in Table 2 for reference. The uE/F ratio, an index of renal HSD11B2 activity, was 1.10 ± 0.41 (range, 0.30-2.70). As shown in Fig. 1, a double reciprocal plot revealed a strong linear correlation between uE and uF (r = 0.810, P < .0001).

uE excretion did not correlate with that of uAld, but uF excretion had a weak positive correlation with that of uAld (r = 0.14, P < .05). In contrast, uE/F ratio had no significant correlation with uAld excretion.

3.2. Relationships between uE, uF, uE/F, or uAld and clinical profiles, serum, or urinary electrolytes

Age correlated with serum levels of Na (r = −0.14, P < .05) or K (r = 0.19, P < .005) and with 24-hour urinary excretions of Na (r = 0.20, P < .005) or K (r = 0.18, P < .01). Diastolic BP had weak negative correlations with uE (r = −0.17, P < .05) and uF (r = −0.15, P < .05). Accordingly, urinary excretion of steroids was adjusted for age and diastolic BP in subsequent analyses.

As summarized in Table 2, uE and uF, but not uE/F, correlated negatively with serum levels of Na and K and positively with 24-hour urinary excretions of Na and K. As expected, uAld correlated negatively with urinary Na excretion and Na/K ratio, but uAld had a rather weak positive correlation with serum K and a negative correlation with K clearance in the present population. uE/F ratio had a weak positive correlation with urinary Na/K ratio but did not correlate with K clearance. uE and uF positively correlated not only with K clearance, but also with urinary Na/K ratio. Furthermore, uE, uF, and uE/F correlated positively with fractional excretions of Na and K, in contrast to a negative correlation of uAld with these fractional excretions. It is noteworthy that uE but not uF negatively correlated with the urinary concentrations of Na and K, and uE/F had stronger negative correlations with both (Figs. 2 and 3).

To elucidate which of the uE/F ratio or uAld preferentially affects urinary concentration of Na and K or urinary Na/K ratio, partial correlation analysis was performed. Pearson's partial correlation coefficients of uE/F for urinary concentrations of Na and K adjusted by uAld in addition to age and diastolic BP were −0.461 and −0.467, respectively, whereas those of uAld adjusted by uE/F were −0.116 and 0.228, respectively. In contrast, the correlation coefficients of urinary Na/K ratio were 0.153 for uE/F and −0.358 for uAld after the corresponding adjustment. Thus, uE/F ratio may have more influence on urinary concentration of Na and K than uAld. In contrast, uAld may contribute more to the urinary Na/K ratio than uE/F ratio.

4. Discussion

Adrenal corticosteroids are metabolized in the liver by reduction to the dihydro or tetrahydro derivatives, followed by conjugation with glucuronides, making them water soluble, and then are excreted in the urine. Here we report an assay for urinary free F and E by using anti-F and anti-E

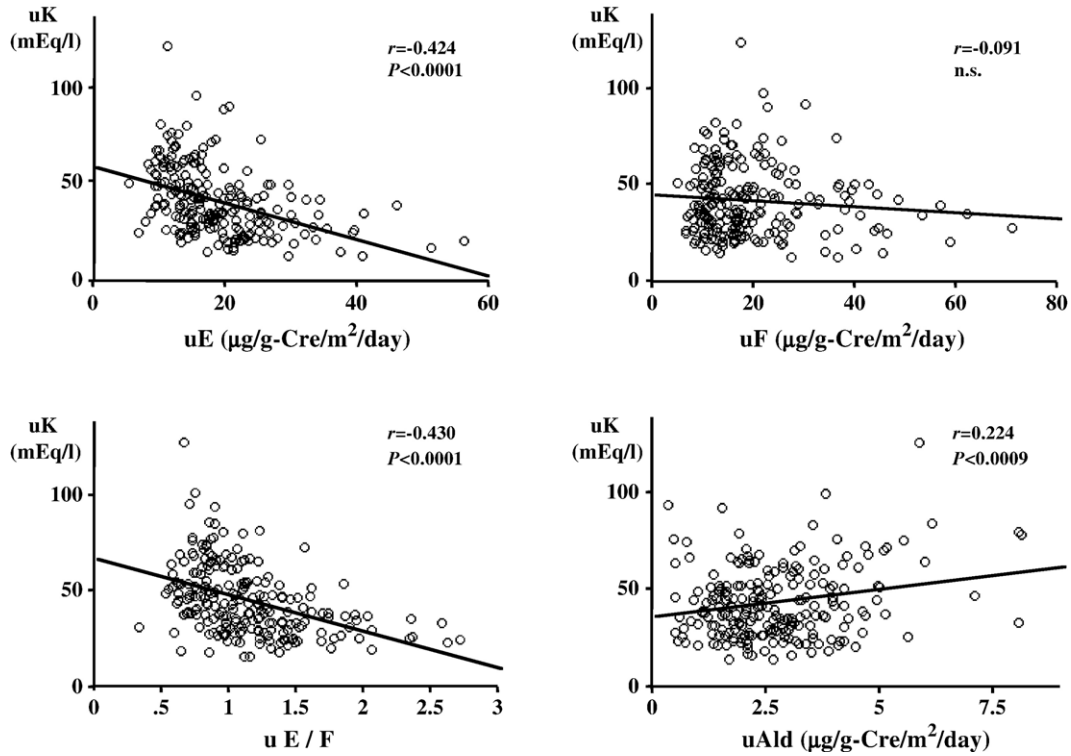

Fig. 3. Correlations between urinary concentration of potassium and uE, uF, uE/F, and uAld. The uE and uE/F had strong negative correlation with urinary concentration of potassium, whereas uF and uAld did not. Data were analyzed by using Pearson's correlation analysis.

antibodies that exhibited high specificity against most other steroids. In the case of the anti-E antibody, a cross-reactivity of 7.5% against 5α-THF was found. However, extraction with methylene chloride separates the urinary free E and F from conjugated steroids [18]. Because tetrahydrometabolites are almost entirely conjugated, they are not extracted with methylene chloride [19], allowing high specificity for the assay. A strong linear correlation between uF and uE in a double reciprocal plot suggests that an enzyme is involved in regulating urinary excretion between these steroids. An estimated K_m value for HSD11B2 deduced from this plot, which is based on a number of individual clinical measurements, was 2.6 μg/dL (72 nmol/L), comparable to the values reported in vitro. The mean uE/F found was 1.10 ± 0.41, which is lower than previously reported values of 1.67 [11] and 1.54 [12]. A decline in renal function with aging can cause a decrease in uE/F ratio [15], but there was no correlation between age and uE/F ratio in the present study. The lower uE/F ratio in our subjects might be explained by difference in the assay method or lower renal HSD11B2 activity in Japanese subjects.

Negative correlations of uAld with urinary Na excretion and Na/K ratio confirm the mineralocorticoid activity of aldosterone, although relationships between serum K, K clearance, and fractional excretions might be difficult to interpret. A weak positive correlation of uE/F ratio with urinary Na/K ratio supports the expected role of renal HSD11B2 as protecting mineralocorticoid receptors for cortisol. However, one of the intriguing findings in our study might be the stronger association of uE and uF with renal electrolyte handling. The positive relationships between urinary excretion of Na or K and uE or uF raise the possibility of separate renal effects mediated by the glucocorticoid receptor. It is established that glucocorticoids increase glomerular filtration rate and have natriuretic and kaliuretic effects, which are independent of the mineralocorticoid receptor [20]. Such a renal glucocorticoid effect might be modulated by functional HSD11B2 in kidney glomerulus [21], implying an additional role of HSD11B2 as modifying access of cortisol to the glucocorticoid receptor.

The present study also revealed stronger negative correlations of uE and uE/F ratio with urinary concentrations of Na and K, compared with uAld. The 24-hour urinary excretion of Na and K is considered to indicate the intake of Na and K. What does the urinary concentration of Na or K mean? As regards the osmolar excretion, urea contributes approximately 50% to the total osmolar excretion, and Na contributes 20% to 30% of the total osmolar excretion in humans [22]. Because urea excretion is constant throughout the day [22], fluctuation of urine osmotic pressure would be mainly determined by the urinary Na concentration, suggesting that urinary Na concentration reflects urine osmotic pressure. In contrast, the rate of K excretion depends on 2 components, the K concentration in the urine and the urine flow rate; each of which is controlled independently. Because there is little variation in flow rate in the human cortical collecting duct, the urinary K concentration would mainly reflect the K concentration in

the terminal cortical collecting duct, which is primarily regulated by the mineralocorticoids [22]. Steele et al [22] suggested that aldosterone does not appear to be the critical factor responsible for minute-to-minute excretion of K, although aldosterone seems to serve a permissive role in minute-to-minute excretion of K. Considering the present strong correlation of uE/F with urinary concentration of Na or K and the diurnal variation in uE/F ratio [23], one can speculate that HSD11B2 might be subjected to rapid response against repletion or depletion of Na or K. Short time changes in uE/F concomitantly with urinary electrolytes and osmolarity should be elucidated, together with plasma arginine vasopressin and adrenocorticotropin.

In conclusion, our study suggests that HSD11B2 is an important regulatory factor of renal sodium and potassium handling independently of and/or complementary to the mineralocorticoid action of aldosterone. The effects of salt loading or depletion on uE/F ratio and electrolyte handling should be studied.

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