ACTIVITY OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 IN NORMOTENSIVE BLACKS AND WHITES

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Background: Salt-sensitive hypertension occurs more commonly in Blacks than in Whites. A decrease in activity of the enzyme 11βHSD2 that results in overstimulation of the mineralocorticoid receptor by cortisol could contribute to greater retention of sodium in Blacks. We tested the hypothesis that less activity of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) is present in Blacks than in Whites.

Methods: Eighty-nine subjects (42 Whites and 47 Blacks), ages 12 to 24 years were recruited from a young cohort that was followed longitudinally in a study of blood pressure regulation. For purposes of study, they were admitted to the General Clinical Research Center. Excretion of tetrahydrocortisol (THF), 5α-THF, and tetrahydrocortisone (THE) was measured in 12-hour overnight urine collections. In vivo 11βHSD2 activity was estimated from the urinary (THF + 5α-THF)/THE) ratio.

Results: Blacks appeared to retain more sodium as evidenced by a lower level of 2-hour upright plasma aldosterone ($P<.001$) and marginally lower plasma renin activity ($P=.06$). The (THF + 5α-THF)/THE ratio in Blacks and Whites was similar: 0.91 ± 0.41 (standard deviation), 0.86 ± 0.52, 1.13 ± 0.36, and 0.66 ± 0.26, in White males, White females, Black males, and Black females, respectively; $P=.35$ for an overall effect of race.

Conclusion: 11βHSD2 activity appears to be similar in Blacks and Whites and probably contributes minimally, if at all, to race differences in sodium retention. (*Ethn Dis*. 2005;15:407–410)

Key Words: 11β-Hydroxysteroid Dehydrogenase Type 2 Enzyme Activity, Blood Pressure, Race, Sodium

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INTRODUCTION

Blood pressure (BP) and extracellular fluid volume are integrally linked to renal sodium handling. Aldosterone plays a central role in the regulation of sodium and water reabsorption in the kidneys, and hence BP. Aldosterone binds to and activates a specific intracellular receptor, the mineralocorticoid receptor (MR), a member of the steroid receptor superfamily.[1] While aldosterone is the preferred ligand for MR, this receptor is also capable of binding and being activated by cortisol to an equal degree.[1] Since the concentration of cortisol in the plasma is two to three orders of magnitude the concentration of the physiologic MR agonist aldosterone,[2] receptor specificity is conferred by the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD),[3] which converts cortisol to its inactive metabolite cortisone in tissues that express the MR, thereby protecting the individual from the consequences of overstimulation of the MR. Two kinetically distinct forms of 11βHSD have been characterized.[4] The low-affinity, nicotinamide adenine dinucleotide phosphate (NADP)-preferring 11βHSD1 is expressed in most tissues and has predominantly reductase activity.[6] In contrast, the high-affinity, nicotinamide adenine dinucleotide (NAD)-requiring 11βHSD2 is preferentially found in cells expressing MR; immunohistochemical studies have consistently localized 11βHSD2 to the distal tubules, and it appears to show only dehydrogenase activity.[5,6] The critical importance of the 11βHSD2 enzyme is underscored by the observation of hypertension in individuals eating licorice (an inhibitor of 11βHSD)[7,8] or with apparent mineralocorticoid excess, a disorder caused by inactivating mutations in the 11βHSD2 gene, where the unprotected MR is overstimulated by cortisol.[9–12]

In the absence of clear-cut inactivation of 11βHSD2 from mutations or inhibitors, we do not know whether a lesser degree of difference in the activity of this enzyme will affect BP. Hypertension is more common in Blacks than Whites[13] and more likely to be salt-sensitive.[14,15] A reduced level of 11βHSD2 activity in Blacks could contribute to the greater sodium retention and the high prevalence of hypertension among Blacks. A previous study showed that certain variant alleles in the 11βHSD2 gene were associated with hypertension in Blacks,[16] no comparison of levels of enzyme activity in Blacks and Whites has been reported. In the present study, we sought to test the hypothesis that Blacks have less 11βHSD2 activity than Whites. We estimated 11βHSD2 activity from the ratio of excreted metabolites of cortisol (tetrahydrocortisol [THF] and 5α-THF) to excreted metabolites of cortisone (tetrahydrocortisone [THE]), with a higher ratio indicating less enzyme activity. Samples were collected from normotensive adolescents and young adults, Blacks and Whites.

METHODS

Study Population and Design

Subjects were recruited from a young cohort that is being followed lon-

Table 1. Characteristics of subjects (mean ± SD)

Characteristic	White Males	White Females	Black Males	Black Females
Number	20	22	20	27
Age (yr)	16.4 ± 3.3	17.9 ± 3.4	15.1 ± 2.4	15.4 ± 2.9
BMI (kg/m²)	25.5 ± 7.6	23.6 ± 4.5	23.5 ± 5.6	25.0 ± 7.0
Supine systolic BP (mm Hg)	112.9 ± 10.0	103.0 ± 5.8	105.9 ± 8.7	105.7 ± 9.2
Supine diastolic BP (mm Hg)	69.0 ± 9.6	64.0 ± 6.1	67.8 ± 10.7	69.9 ± 9.7
Supine PRA (ng/L/s)	0.48 ± 0.32	0.35 ± 0.30	0.30 ± 0.18	0.31 ± 0.27
Upright PRA (ng/L/s)	1.89 ± 0.98	1.50 ± 1.06	1.48 ± 1.36	1.38 ± 1.10
Supine aldosterone (pmol/L)	341.2 ± 176.2	290.9 ± 265.2	240.7 ± 177.5	239.5 ± 209.3
Upright aldosterone (pmol/L)	921.6 ± 286.3	1135.3 ± 574.7	640.1 ± 436.0	836.7 ± 617.6
THF (μg/12 hr)	509.4 ± 371.3	292.5 ± 116.1	305.8 ± 163.6	276.7 ± 133.3
THF-5α (μg/12 hr)	707.3 ± 515.9	318.9 ± 196.7	466.5 ± 356.8	281.0 ± 175.5
THE (μg/12 hr)	1339.2 ± 785.0	711.7 ± 328.2	684.7 ± 319.5	846.4 ± 442.1
(THF+THF-5α)/THE	0.91 ± 0.41	0.86 ± 0.52	1.13 ± 0.36	0.66 ± 0.26

Supine blood pressure (BP), PRA, and aldosterone was measured at 7 AM before the patients arose from bed after an overnight sleep. Upright values were measured 2 hours after rising from bed.

BP = blood pressure; PRA = plasma renin activity; THF = urinary tetrahydrocortisol; THF-5α = urinary tetrahydro 5α cortisol; THE = urinary tetrahydrocortisone.

gitudinally in a study of BP regulation and were requested by letter to participate in this study. Of those contacted, 129 volunteered to participate in the study. After exclusion of siblings and participants with incomplete information, 89 individuals were included in the study. The characteristics of the subjects are described in Table 1. Of the 89 subjects, 40 were males (20 White and 20 Black), and 49 were females (22 White and 27 Black). All were in good health, and none was taking medications that could affect sodium retention or BP. Pregnancy was excluded in the female participants. The institutional review board (IRB) of the Indiana University School of Medicine approved the study protocol, and all the procedures followed were in accordance with institutional guidelines.

After obtaining informed consent, the participants were admitted to the General Clinical Research Center in the afternoon. A 12-hour, overnight urine sample (from 7 PM to 7 AM) was obtained from each subject to measure urinary levels of THF, 5α-THF, and THE. The next morning, BP, plasma renin activity (PRA), and plasma aldosterone levels were measured while the subjects were still supine and were repeated after one hour of sitting and one hour of standing. Blood pressure (BP) was measured by using a mercury sphygmomanometer, and the average of three readings was used in the analysis.

Analytical Methods

Plasma aldosterone was measured by radioimmunoassay (RIA) with antiserum from Diagnostic Products Corporation (Los Angeles, Calif) with values expressed as pmol/L. Plasma renin activity (PRA) was measured with the Clinical Assays GammaCoat RIA kit (Diasorin, Inc; Stillwater, Minn.) and the values are expressed as ng/L/s. Urinary levels of THF, 5α-THF, and THE were analyzed by gas chromatography-mass spectrometry on a Hewlett-Packard gas chromatograph 6890 (Palo Alto, Calif.) equipped with a mass selective detector 5973 as previously described.[8] The activity of the 11βHSD2 enzyme was assessed by the (THF+5α-THF)/THE ratio, with a lower ratio indicating higher 11βHSD2 activity.

Statistical Analyses

Data are presented as mean ± standard deviation (SD) unless otherwise noted. Comparisons between race and sex groups were made by using two-way analysis of variance to examine for a race-sex interaction. When the interaction was not significant, the race and sex comparisons were made by using two-sample *t* tests. Spearman's rank correlation coefficient was used to assess bivariate relationships. Analysis of variance was also used to prediction BP by the (THF+5α-THF)/THE ratio, with age, body mass index (BMI—weight in kg/[height in m]²), sex, race, and sex-by-race interaction as predictors. For PRA, aldosterone, THF, 5α-THF, THE, and (THF+5α-THF)/THE, we used the logarithm transformation before calculating significance, but the original units are reported in Table 1.

RESULTS

The characteristics of the subjects are presented in Table 1. The Whites (n=42) and Blacks (n=47) ranged from 12 to 24 years in age; the Whites were slightly but significantly older than the Blacks (P=.0041). Body mass index (BMI) was comparable between the race and sex groups (Table 1). Supine systolic BP (obtained at 7:00 AM after having been recumbent overnight) was significantly higher in the White males compared to the White females (P=.0004), the Black males (P=.0122), and the Black females (P=.0061); no other sig-

nificant differences were seen in supine systolic BP between groups ($P>.27$). No differences between groups were found with respect to the supine diastolic BP. After two hours of being upright, the BPs increased with no difference between any of the groups (data not shown). As expected, PRA and aldosterone levels were lower when the subjects were supine. No statistically significant differences were seen between the males and females in these parameters either in the supine position or after two hours of being upright. However, a significant race effect was seen on plasma aldosterone levels two hours after rising, with the Blacks having significantly lower ($P=.0008$) aldosterone levels than the Whites. A marginally significant race effect was seen on PRA supine ($P=.06$) and two hours after rising ($P=.10$), with Blacks again having lower levels than Whites.

In the females, urinary excretion rates of THF ($P=.06$) and 5α-THF ($P=.0016$) were lower than in the males. A marginally significant race effect was seen for THF ($P=.07$), with the Whites having higher values than the Blacks, race was not related to the excretion of 5α-THF ($P=.15$). The excretion of THE was significantly higher in the White males than in the White females ($P=.0036$), the Black males ($P=.0027$), and the Black females ($P=.0367$); no other relationship of THE was found to race or sex ($P>.23$).

The (THF+5α-THF)/THE ratio was significantly lower in the Black females than in the Black males ($P=.0001$), White females ($P=.0120$), and White males ($P=.0122$), but no other difference were seen in the ratio between groups (Table 1). The (THF+5α-THF)/THE ratio was unrelated to BMI or age overall or in any subgroup ($P>.09$). No significant relationship was found between the (THF+5α-THF)/THE ratio and BP, PRA (both while supine after 12 hours of recumbency and 2 hours after being upright), or the supine plasma aldosterone level in either race or sex group (all $P>.39$). A marginally significant inverse relationship of the (THF+5α-THF)/THE ratio was seen to the upright plasma aldosterone level in the males ($P=.07$), Whites ($P=.10$), and when all subjects were combined ($P=.08$). No significant relationship was found of (THF+5αTHF)/THE ratio to serum potassium concentration as might be expected with a difference in 11βHSD2 activity. No relationship of enzyme activity level to the 12 hour urinary sodium excretion rate was found.

DISCUSSION

In the present study, no significant racial difference was seen in the (THF+5α-THF)/THE ratio, an index of 11βHSD2 activity. The findings suggest that the activity of this enzyme in the kidney may not be an important determinant of the difference in salt and water retention in Blacks and Whites. The subjects participating in this study appeared to be representative of racial groups where a difference in sodium retention has been reported: the two-hour upright plasma aldosterone concentration was lower ($P<.001$) and levels of PRA were marginally lower ($P=.06$) in the Blacks than in the Whites, consistent with more sodium retention in the Blacks.

A lack of demonstration of a relationship of 11βHSD2 activity to race, or for that matter the levels of renin activity and aldosterone, could result from a sample size that was statistically underpowered to detect such a difference. The subjects were, however, admitted to an inpatient facility (GCRC) where measurements could be carried out under carefully controlled conditions, thereby reducing the need for study of very large samples. It would seem that at most we would have missed a small race effect. Our subjects were studied under basal conditions and it is possible that had we performed a perturbation of sodium balance that a difference between groups with respect to 11βHSD2 activity might have been delineated. For example, a previous study identified an association of a genetically determined decrease in 11βHSD2 activity with an enhanced BP response to salt loading in young White males.[17]

The ratio of (THF + 5α-TF) and THE in the urine has been used to estimate the activity of the enzyme 11βHSD2, with a lower ratio indicating higher activity of the enzyme. The same ratio has been used to measure the activity of the type 1 isoform of the enzyme, with a lower ratio indicating higher activity. Nonetheless, the (THF+5α-THF)/THE ratio has been shown to be a sensitive and reproducible parameter for the assessment of normal or decreased 11βHSD2 activity in vivo,[18] although we do not know if this parameter is sufficiently sensitive to differentiate increased 11βHSD2 oxidative activity from decreased 11βHSD1 reductase activity. In either case, a low ratio reflects relatively lower levels of cortisol and higher levels of cortisone, which should result in better protection of the MR in individuals with a lower ratio. If this factor were important in determining salt and water balance, individuals with a lower ratio should have lower BP.

In previous studies, we showed that the principal mineralocorticoid, aldosterone, was lower in Blacks than in Whites.[19] We also found that levels of other mineralocorticoids were either lower in Blacks or similar in Blacks and Whites.[20] These earlier data together with those presented here are more suggestive of a primary renal mechanism for the increased sodium retention in Blacks, such as molecular variations in sodium transporters per se.

ACKNOWLEDGMENTS

Studies were supported by NIH grants R01-HL-35795 and 5 R01 HL067360, a merit review grant from the US Department of Veterans Affairs, and a Nicholas H. Noyes,

Jr., Memorial Foundation grant (JHP); an NIH grant KO8 DK59831-01 (RRS); the Swiss National Foundation for Scientific Research (Nr. 3100-58889) and the Cloëtta Foundation, Zurich, Switzerland (FP); and the NIH grant M01-RR00750.

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