J. steroid Biochem. Vol. 27, No. 4–6, pp. 971–975, 1987
Printed in Great Britain.

0022-4731/87 $3.00 + 0.00

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

ROLE OF 18-HYDROXYLATED CORTISOLS IN HYPERTENSION

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Summary—The isolation of 18-hydroxycortisol and 18-oxocortisol was recently described. These steroids have been shown to be excreted in exaggerated quantities in patients with primary aldosteronism, with adrenal adenomas and in glucocorticoid suppressible aldosteronism. We report the measurement of both steroids in the urine of patients with essential hypertension. 18-Oxocortisol excretion did not differ in patients with normal renin essential hypertension (0.7 ± 0.7 μg/24 h), low renine essential hypertension (0.7 ± 0.5 μg/24 h) and normal individuals (1.2 ± 0.9 μg/24 h). Patients with normal renin hypertension excreted 54 ± 43 μg/24 h of 18-hydroxycortisol, those with low renin essential hypertension excreted 58 ± 54 μg/24 h, and normal individuals excreted 63 ± 36 μg/24 h. Three of the low renin and one of the normal renin hypertensive subjects excreted greater quantities of 18-hydroxycortisol than the upper limit of normal, but all excreted normal quantities of 18-oxocortisol. As 18-hydroxycortisol is inactive, the meaning of this elevated excretion is unclear, but it may be a marker of an adrenal enzymatic abnormality which may be playing a more direct role in hypertension.

Adrenocorticoid steroids play a clear role in diseases like primary aldosteronism, Cushing's syndrome, 11β- and 17-hydroxylase deficiencies and very probably in rare cases of patients with low renin hypertension [1–5]. They are also suspected of playing a role in some forms of essential hypertension [1]. Patients with primary aldosteronism frequently present with a syndrome where the severity of the metabolic abnormality and level of hypertension is out of proportion to the degree of abnormality in aldosterone secretion [3, 6]. Similary, there are many cases of patients with low renin hypertension in which the metabolic syndrome strongly suggests the possibility that an adrenal secretory product with mineralocorticoid activity is produced in excess, but measurements of the known mineralocorticoids have not been shown to be increased. This has led to the postulate that an unidentified mineralocorticoid is being produced [4, 5]. In the course of studies of steroids excreted in the urine of patients with primary aldosteronism, a previously unknown derivative of cortisol was isolated and identified [6]. This steroid, 18-hydroxycortisol, was found to be a major steroid excreted in the urine of patients with primary aldosteronism, particularly in those patients with adrenal adenomas and in glucocorticoid suppressible aldosteronism (GSA) [6]. Incubations of bullfrog interrenal tissue, a model of zona glomerulosa, showed that cortisol could be metabolized through the same pathway by which corticosterone is transformed to 18-hydroxycorticosterone and aldosterone. Cortisol acts as a suboptimal substrate for the cytochrome P-450 corticosterone methyl oxidase producing 18-hydroxy-cortisol and 18-oxocortisol [7]. Studies from this laboratory have shown that the excretion of 18-oxocortisol is increased markedly in patients with glucocorticoid suppressible aldosteronism and moderately in those with primary aldosteronism with adrenal adenomas [8].

The role of excess production of 18-hydroxylated cortisols (18-hydroxy and oxo) in primary aldosteronism is not clear. The biological activity of 18-hydroxycortisol as a gluco- or mineralocorticoid is neglible [9], but 18-oxocortisol has 1% the salt-retaining activity of aldosterone and 3% the glucocorticoid activity of cortisol in *in vivo* and *in vitro* bioassays [10, 11]. The chronic injection of 18-oxocortisol into rats induces hypertension [12]. It is possible that 18-oxocortisol plays a role in the pathogenesis of hypertension in those cases where the production is markedly increased. 18-Hydroxy and 18-oxocortisol may also be useful as a marker in the differentiation of patients with the various types of primary aldosteronism [8, 13].

We are presenting our studies of the excretion of 18-hydroxycortisol and 18-oxocortisol in patients with essential hypertension and the correlation of the excretion of these steroids with that of aldosterone 18-oxoglucuronide, free cortisol and 19-nordeoxycorticosterone.

METHODS

Radioimmunoassays. The urinary excretion of 18-oxocortisol, 19-nordeoxycorticosterone, cortisol and 18-hydroxycortisol was measured by radioimmunoassay as previously described [8, 14–17].

Experimental subjects. Forty-seven subjects with mild to moderate established hypertension were studied while consuming a normal outpatient diet containing at least 100 mmol of sodium. The sub-

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Table 1. Urinary excretion in hypertensive subjects

	Normals ($n = 37$)	Hypertensives NRH ($n = 17$)	LRH ($n = 30$)
18-Hydroxycortisol	63 ± 36 μg/24 h	54 ± 43	58 ± 54
18-Oxocortisol	1.2 ± 0.9 μg/24 h	0.7 ± 0.7	0.7 ± 0.5
Aldosterone 18-oxo G	6.1 ± 2.7 μg/24 h	6.7 ± 3.4	6.5 ± 4.7
19-Nordeoxycorticosterone	313 ± 268 ng/24 h	235 ± 170	224 ± 169
Cortisol	10 ± 10 μg/24 h	22 ± 16	30 ± 50

jects collected a 24-h urine while being off all medications for at least 2 weeks. The patients were classified according to their renin status, using the furosemide test as previously described [18]. For comparison, 37 normal individuals were studied under similar conditions.

RESULTS

The urinary excretion of 18-hydroxycortisol in 37 normal subjects using a HPLC-RIA was 63 ± 36 (SD) μg/24 h. Thirty subjects that had low renin hypertension excreted 58 ± 54 μg/24 h and 17 with normal renin hypertension excreted 54 ± 43 μg/24 h. Three of the LRH group and one of the NRH group excreted more than 2 SD (135 μg/24 h) above the mean of the normals.

The urinary excretion of 18-oxocortisol in the 37 normal subjects was 1.2 ± 0.9 μg/24 h (SD) with 2 SD being 3.0 μg/24 h. None of the hypertensive subjects excreted greater quantities than the normal limits. The four subjects that excreted increased quantities of 18-hydroxycortisol excreted normal amounts of 18-oxocortisol and the other steroids measured. The results are shown in Fig. 1 and Table 1.

DISCUSSION

The postulated biosynthetic pathway of 18-oxocortisol and 18-hydroxycortisol is shown in Fig. 2. Cortisol is metabolized by the cytochrome P-450 corticosterone methyl oxidase by two sequential hydoxylations. The first hydroxylation at the 18-position yields 18-hydroxycortisol, which is then hydroxylated again to produce 18-oxocortisol [7]. Definite proof of this sequence is still not available, but it is the best assumption to explain the conversion to these 17-hydroxylated analogs of 18-hydroxycorticosterone and aldosterone in bullfrog adrenal homogenates. We have previously shown that 11-deoxycortisol can follow a different pathway in its conversion to 18-hydroxycortisol by undergoing an initial 18-hydroxylation to produce 18-hydroxy-11-deoxycortisol, which in turn is 11-hydroxylated to yield 18-hydroxycortisol [19]. This synthesis can occur not only in the zona glomerulosa (or more likely in the transitional zone), but also in the zona fasciculata, which lacks the cytochrome P-450 corticosterone methyl oxidase [20]. Adrenal cells originate at the subcapsular area and undergo a series of morphologic and functional changes as they migrate centripetally from the zona glomerulosa to the fasciculata-reticularis [21]. The cytochrome P-450 corticosterone methyl oxidase undergoes involution as the cells become fasciculata, and, under the action of ACTH, there is the induction of the 17-hydroxylase enzyme, resulting in the synthesis of cortisol. The induction of the 17-hydroxylase seems to occur in the outer fasciculata [20, 22]. We have suggested that in the interphase of the zona glomerulosa and fasciculata, which we have called the transitional zone, there are cells which contain both enzymes,

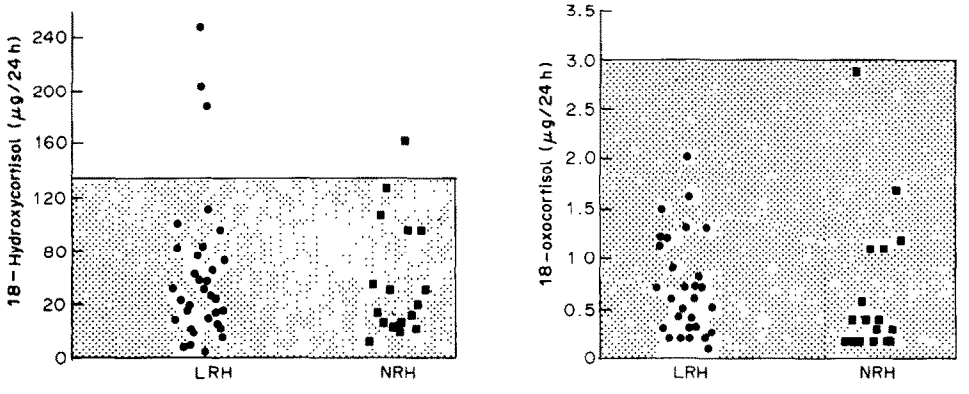

Fig. 1. Excretion of 18-hydroxycortisol and 18-oxocortisol in patients with essential hypertension. The shaded area represent the normal limits.

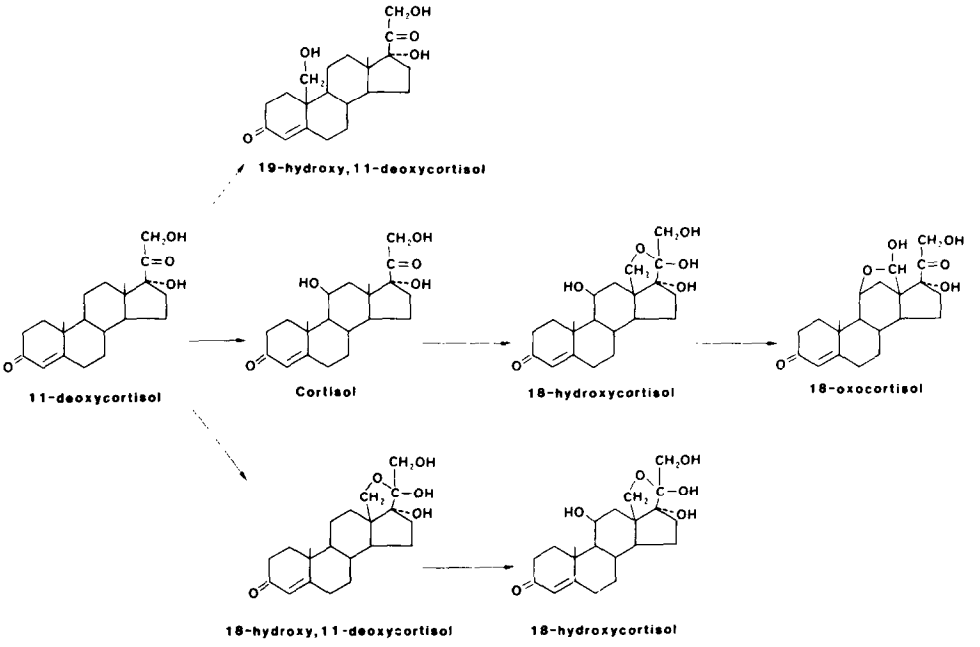

Fig. 2. Metabolic conversion of 11-deoxycortisol in the adrenal.

providing the opportunity for the synthesis of these hybrid steroids. Detailed studies on enzymatic localization have yet to be reported.

It is very unlikely that 18-oxocortisol plays a role in the pathogenesis of patients with essential hypertension. The excretion of 18-oxocortisol is entirely normal, and the relatively weak mineralo- and glucocorticoid activity of this compound [10, 11] would mandate that the abnormality had to be severe for it to be involved in hypertension. In patients with primary aldosteronism, either with adrenal adenomas or with glucocorticoid suppression aldosteronism, 18-oxocortisol is excreted in greatly exaggerated quantities and could be important in the pathogenesis of hypertension. Plasma from patients with glucocorticoid suppressible aldosteronism contains a greater quantity of mineralocorticoid activity as measured by the plasma mineralocorticoid receptor assay than can be explained by the simultaneous measurement of aldosterone, cortisol and deoxycorticosterone and the estimation of their contribution to the measured plasma mineralocorticoid receptor activity [23, 24]. We have previously postulated that the degree of abnormality in the production of 18-oxocortisol in these patients makes it likely that the unknown contributor to the increased plasma mineralocorticoid receptor activity is 18-oxocortisol [8].

The explanation as to why a small subset of patients with hypertension, irrespective of their renin status, excrete greater quantities of 18-hydroxycortisol is not apparent. The finding that the excretion of aldosterone or 18-oxocortisol is normal suggests that there is no increase in the activity of the enzyme cytochrome P-450 corticosterone methyl oxidase in the zona glomerulosa. As shown in Fig. 2, 18-hydroxycortisol can also be synthesized via the cytochrome P-450 11,18-hydroxylase path. By this route the end-product 18-hydroxycortisol does not seem to undergo further conversion and is secreted as such from the adrenal. It is possible that an increased activity of the 18-hydroxylase component might be responsible for the increased production of 18-hydroxycortisol. This type of defect has been seen in Dahl S and R hypertensive rats. Dahl created two different strains of rats by selective breeding for sensitivity (S rat) or resistance (R rat) to the hypertensinogenic effects of an increased salt intake [25]. The Dahl S rat adrenal produces more 18-hydroxydeoxycorticosterone compared to the R rat. It has been estimated that 16% of the elevated blood pressure is due to this adrenal abnormality [26]. Enzymatic studies with rat adrenals have shown that 18-hydroxylation is greater in the S rat compared to the R rat, and this most likely explains the increased production and plasma concentrations of 18-hydroxydeoxycorticosterone in the S strain [27]. Because the biological activity of 18-hydroxydeoxycorticosterone is low [28], this abnormality may be an incidental marker for an enzymatic abberation and not be playing a direct or important role in the hypertension, in which case the exact adrenal cause of the hypertension remains unclear. It is possible that the same situation occurs in the occasional

human who exhibits an increased secretion of 18-hydroxycortisol. 18-Hydroxydeoxycorticosterone levels have been found to be abnormally high in certain hypertensive people [4], although the marked alterations initially reported have not been confirmed [29]. Subtle abnormalities in plasma 18-hydroxydeoxycorticosterone concentrations have also been well documented [30]. It is possible that in the human, where 17-hydroxylated steroids predominate, measurements of metabolic products of the 11,18-hydroxylase on a 17-hydroxylated precursor would be a more sensitive method to detect an abnormality similar to that in the Dahl S rat. The measurement of 18-hydroxycortisol would give an indication of such an enzyme abnormality, but it would be better to measure the predecessor, 18-hydroxy-11-deoxycortisol. Unfortunately, no methods for its synthesis or quantification have been described. If either compound is found to be produced in increased quantities, their low biological activity probably indicates that they serve as marker steroids for the abnormality rather than as part of the pathogenesis of the hypertension.

Acknowledgements—The expert technical help of Mark F. Foecking, Robert J. Upcavage and Melinda Lake is gratefully acknowledged. These studies were supported in part by Medical Research Funds from the Veterans Administration, NIH grants (HL 27255 and 26856) and by grant RR-73 from the General Clinical Research Centers Program of the Division of Research Resources, NIH.

REFERENCES

1. Gomez Sanchez C. E.: The role of steroids in human essential hypertension. *Biochem. Pharmac.* **31** (1982) 893–897.
2. Gomez-Sanchez C. E.: Cushing's syndrome and hypertension. *Hypertension* **8** (1986) 258–264.
3. Ulick S.: Adrenocortical factors in hypertension—I. Significance of 18-hydroxy-11-deoxycorticosterone. *Am. J. Card.* **38** (1976) 814–824.
4. Melby J. C. and Dale S. L.: Adrenal steroidogenesis in "low renin" or hyporeninemic hypertension. *J. steroid Biochem.* **6** (1976) 761–766.
5. Skrabal F.: Aldosterone and *in vivo* mineralocorticoid activity in normotensive and hypertensive man. *J. R. Soc. Med.* **72** (1979) 252–259.
6. Chu M. D. and Ulick S.: Isolation and identification of 18-hydroxycortisol from the urine of patients with primary aldosteronism. *J. biol. Chem.* **258** (1982) 2218–2224.
7. Ulick S., Chu M. D. and Land M.: Biosynthesis of 18-oxocortisol by aldosterone producing adrenal tissue. *J. biol. Chem.* **258** (1983) 5498–5502.
8. Gomez-Sanchez C. E., Montgomery M., Ganguly A., Holland O. B., Gomez-Sanchez E. P., Grim C. E. and Weinberger M. H.: Elevated urinary 18-oxocortisol in glucocorticoid-suppressible aldosteronism. *J. clin. Endocr. Metab.* **59** (1984) 1022–1024.
9. Gomez-Sanchez E. P., Gomez-Sanchez C. E., Smith J. S., Ferris M. W. and Foecking M. F.: Receptor binding of biological activity of 18-hydroxycortisol. *Endocrinology* **115** (1984) 462–466.
10. Gomez-Sanchez C. E., Gomez-Sanchez E. P., Smith J. S., Ferris M. W. and Foecking M. F.: Receptor binding and biological activity of 18-oxocortisol. *Endocrinology* **116** (1985) 6–10.
11. Ulick S., Land M. and Chu M. D.: 18-Oxocortisol, a naturally occurring mineralocorticoid agonist. *Endocrinology* **113** (1983) 2320–2322.
12. Hall C. E. and Gomez-Sanchez C. E.: Hypertensive potency of 18-oxocortisol in the rat. *Hypertension* **8** (1986) 317–322.
13. Ulick S. and Chu M. D.: Hypersecretion of a new corticosteroid, 18-hydroxycortisol in two types of adrenocortical hypertension. *Clin. exp. Hyper.* **A4** (1982) 1771–1777.
14. Gomez-Sanchez C. E. and Holland O. B.: Urinary tetrahydroaldosterone and aldosterone 18-oxoglucuronide excretion in white and black normal subjects and hypertensive patients. *J. clin. Endocr. Metab.* **52** (1981) 214–219.
15. Schoneshofer M., Fenner A., Attinok G. and Dulce B. H.: Cortisol by combination of automatic high pressure liquid chromatography and radioimmunoassay. *Clin. Acta* **106** (1980) 63–73.
16. Gomez-Sanchez C. E., Holland O. B. and Upcavage R.: Urinary free 19-nordeoxycorticosterone and deoxycorticosterone in human hypertension. *J. clin. Endocr. Meta.* **60** (1985) 234–238.
17. Gomez-Sanchez C. E., Upcavage R. J., Zager P., Foecking M. F., Holland O. B. and Ganguly A.: Urinary 18-hydroxycortisol and its relationship to the excretion of other adrenal steroids. *J. clin. Endocr. Metab.* (1987) In press.
18. Kaplan N. M., Kem D. C., Holland O. B., Kramer N. J., Higgins J. and Gomez-Sanchez C.: The intravenous furosemide test: a simple way to evaluate renin responsiveness. *Ann. intern. Med.* **84** (1976) 639–645.
19. Gomez-Sanchez C. E., Foecking M. F., Shackleton C. H. L., Chavarri M. L. and Gomez-Sanchez E. P.: 18-Hydroxy-11-deoxycortisol: a new steroid isolated from incubations of adrenals with 11-deoxycortisol. *J. steroid Biochem.* **26** (1986) 105–111.
20. Gomez-Sanchez C. E.: 18-Hydroxycortisol and 18-oxocortisol, steroids from the transitional zone. *Endocr. Res.* **10** (1984–5) 609–615.
21. Bertholet J. Y.: Proliferative activity and cell migration in the adrenal cortex of fetal and neonatal rats: an autoradiographic study. *J. Endocr.* **87** (1980) 1–9.
22. Crivello J. F. and Gill G. N.: Induction of cultured bovine adrenocortical zona glomerulosa cell 17 hydroxylase activity by ACTH. *Mol. cell Endocr.* **30** (1983) 97–107.
23. Lan N. C., Matulich D. T., Stockigt J. R., Biglieri E. G., New M. I., Winter J. S. D., McKenzie J. K. and Baxter J. D.: Radioreceptor assay of plasma mineralocorticoid activity. Role of aldosterone, cortisol and deoxycorticosterone in various mineralocorticoid-excess states. *Circulat. Res.* **46** (1980) I-94–I-100.
24. Speiser P. W., Martin K. O., Kao-Lo G. and New M. I.: Excess mineralocorticoid receptor activity in patients with dexamethasone-suppressible hyperaldosteronism is under adrenocorticotropin control. *J. clin. Endocr. Metab.* **61** (1985) 297–302.
25. Dahl L. K., Heine M. and Tassinari L.: Effects of chronic excess salt ingestion: evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J. exp. Med.* **115** (1963) 1173–1190.
26. Rapp J. P. and Dahl L. K.: Mendelian inheritance of 18 and 11B-steroid hydroxylase activities in the adrenals of rats genetically susceptible or resistant to hypertension. *Endocrinology* **90** (1972) 1435–1446.
27. Rapp J. P. and Dahl L. K.: Adrenal steroidogenesis in rats bred for susceptibility and resistance to the hypertensive effect of salt. *Endocrinology* **88** (1971) 52–65.
28. Hall C. E., Gomez-Sanchez C. E., Holland O. B., Nasseth D. and Hall O.: Experimental hypertension

and other responses to 18-hydroxy-deoxycorticosterone treatment in the rat. *Endocrinology* **103** (1978) 133–140.
29. Agrin R. J., Dale S. L., Holbrook M., LaRossa J. T. and Melby J. C.: Urinary free 18-hydroxy-11-desoxycorticosterone excretion in normal and hypertensive patients. *J. clin. Endocr. Metab.* **47** (1978) 877–884.
30. Williams G. H., Braley L. M. and Underwood R. H.: The regulation of plasma 18-hydroxy-11-deoxycorticosterone in man. *J. clin. Invest.* **58** (1976) 212–229.