Pergamon

0960-0760(94)E0008-9

J. Steroid Biochem. Molec. Biol. Vol. 49, No. 1, pp. 69–71, 1994

0960-0760/94 $7.00 + 0.00

Biosynthetic Pathway of 19-Noraldosterone in Isolated Rat Glomerulosa Cells

Yoshiyu Takeda,* Isamu Miyamori, Kazuhiro Iki, Takashi Yoneda and Ryoyu Takeda

Second Department of Internal Medicine, School of Medicine, Kanazawa University, Takara-machi, Kanazawa 920, Japan

The biosynthetic pathway of 19-noraldosterone (19-noraldo) in isolated rat glomerulosa cells (GC) and fasciculata-reticular cells (FC) was studied by analyzing [^{14}C]pregnenolone metabolism using HPLC and quantification by specific RIA. In GCs, 18,19-dihydroxycorticosterone was detected after 15 min incubation with [^{14}C]pregnenolone, 18-hydroxy-19-norcorticosterone was detected after 30 min and 19-noraldo was detected after 45 min before the appearance of an aldosterone peak. These three mineralocorticoids were not detected in FCs. The results demonstrate that 19-noraldo is synthesized in GCs and then undergoes further metabolism.

J. Steroid Biochem. Molec. Biol., Vol. 49, No. 1, pp. 69–71, 1994

INTRODUCTION

19-Noraldosterone (19-noraldo) possesses potent mineralocorticoid activity and hypertensinogenic potency [1, 2]. 18,19-Dihydroxycorticosterone [18,19(OH)$_2$-B] and 18-hydroxy-19-norcorticosterone (18-OH-19-nor-B), a possible precursor of 19-noraldo, are hypertensinogenic in rats [3, 4]. These mineralocorticoids were detected in human urine [5–7]. Synthesis of 19-noraldo was elevated in patients with aldosterone-producing adenomas [8]. We studied [^{14}C]pregnenolone metabolism in isolated rat glomerulosa cells (GC) and fasciculata-reticular cells (FC) to clarify the biosynthetic pathway of 19-noraldo.

EXPERIMENTAL

Steroids

Commercially available unlabeled steroids were purchased from Sigma (Tokyo, Japan): [^{14}C]pregnenolone was purchased from New England Nuclear (Tokyo, Japan). 18,19(OH)$_2$-B, 18-OH-19-nor-B and 19-noraldo were obtained from the Department of Biotechnology (Tel Aviv University, Israel).

Preparation of cell suspensions and incubation

Male Wistar rats (180–200 g in weight) were purchased from Nippon Charles River Labs (Kanagawa, Japan) and maintained on a normal sodium diet. They were killed by decapitation, the adrenals were removed immediately and collected in ice-cold normal saline. Loose connective tissue and fat clinging to the adrenals were removed under a dissecting microscope. The adrenal glands were bisected and separated into capsular and decapsulated portions [9]. After rat GCs and FCs were preincubated in 2 ml Krebs-Ringer's solution, pH 7.4, containing 0.2% glucose and serum albumin (0.2%) (KRBSA) at 37°C in an atmosphere of 95% oxygen and 5% carbon dioxide. After 1 h the preincubation buffer was replaced with [^{14}C]pregnenolone or with control buffer and incubation continued for 15, 30, 45, 60 or 120 min. The medium was centrifuged for 30 min at 300 rpm at each time point.

Steroid analysis

Radioactivity in the incubation medium containing [^{14}C]pregnenolone was extracted using a Sep-pak C18 cartridge (Waters Associates, Milford, MA) and the extracts were subjected to reverse-phase HPLC.

HPLC analysis. The analytical column (C-18 Ultrasphere ODS column, 5 μm, Beckman, Tokyo, Japan) was treated with a solvent system: water–acetonitrile–methanol (72:23:5, by vol) at a flow rate of 2.0 ml/min. Retention times of 18,19(OH)$_2$-B, 18-OH-19-nor-B, 19-noraldo, 18-hydroxycorticosterone (18-OH-B), aldosterone and pregnenolone were 11, 16, 20, 24, 28 and 60 min, respectively. Samples of each steroid fraction were added to 5 ml of scintillation liquid and

*Correspondence to Y. Takeda.
Received 28 July 1993; accepted 23 Dec. 1993.

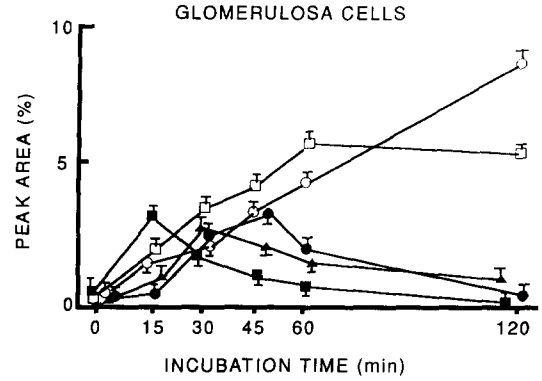

Fig. 1. Time course for the formation and subsequent metabolism of 19-noraldo by adrenal zona glomerulosa cells. The cells were incubated with 20 nM of [^{14}C]pregnenolone. (●) 19-noraldo, (■) 18,19(OH)$_2$-B, (▲) 18-OH-19-nor-B, (□) 18-OH-B, (○) aldosterone.

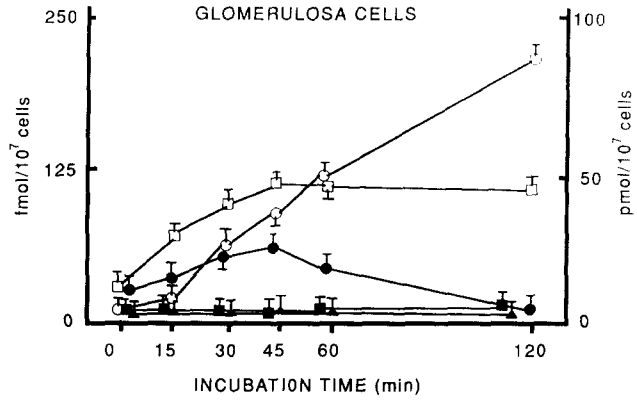

Fig. 3. RIA determination of each steroid after separation by HPLC. 19-noraldo (●), 18,19(OH)$_2$-B (■) and 18-OH-19-nor-B (▲) (fmol/10^7 cells). 18-OH-B (□) and aldosterone (○) (pmol/10^7 cells).

radioactivity was counted in an Aloka LSC 3500 scintillation counter (Tokyo, Japan). "Peak area" was calculated as percentage of radioactivity of each steroid fraction per total count of [^{14}C]pregnenolone. Samples of each steroid obtained from the incubation medium without [^{14}C]pregnenolone were extracted similarly with a Sep-pak C18 cartridge and evaporated under a stream of nitrogen gas. The steroid content was measured by RIA specific for each steroid [7, 10].

All experiments were done in triplicate. The data were expressed as means ± SEM.

RESULTS

19-Noraldo was detected in the GC incubation medium using HPLC-MS. The time course for formation and disappearance of 18,19(OH)$_2$-B, 18-OH-19-nor-B, 19-noraldo, 18-OH-B and aldosterone in rat GC is illustrated in Fig. 1. 19-Noraldo was first noted at 30 min, showed a maximal peak at 45 min, and was then further metabolized. In FCs, there was no 19-noraldo peak (Fig. 2), but small peaks of 18-OH-B and aldosterone, which were caused by cross-contamination between the cells, were observed. 19-Noraldo was not detectable by RIA in FCs. The steroid content in GCs confirmed that 19-noraldo was only synthesized in GCs (Fig. 3).

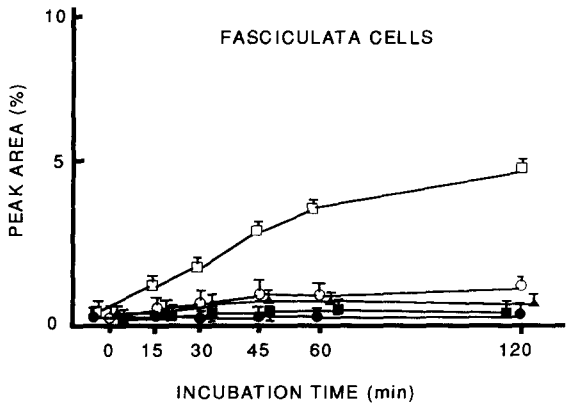

Fig. 2. Time course for the formation and subsequent metabolism of 19-noraldo by adrenal FC cells. The cells were incubated with 20 nM of [^{14}C]pregnenolone. (●) 19-noraldo, (■) 18,19(OH)$_2$-B, (▲) 18-OH-19-nor-B, (□) 18-OH-B, (○) aldosterone.

DISCUSSION

The 19-nor-steroid derivatives possess potent mineralocorticoid and hypertensinogenic activity [11, 12]. Urinary 19-noraldo, 18,19(OH)$_2$-B and 18-OH-19-nor-B excretion is increased in patients with primary aldosteronism [10]. We previously reported that 19-noraldo, 18,19(OH)$_2$-B and 18-OH-19-nor-B were produced by both normal adrenal tissue and aldosterone-producing adenomas [8]. Sodium restriction increases urinary excretion of 19-noraldo and 18,19(OH)$_2$-B, suggesting that these mineralocorticoids are, in part, controlled by the renin–angiotensin system [13]. The present results show that like aldosterone, 19-noraldo is synthesized in adrenal glomerulosa cells. Aldosterone has been found to be synthesized by cytochrome *P*450aldo from zona glomerulosa mitochondria of rat adrenal cortex [14]. The cDNA of cytochrome *P*450aldo has been cloned and sequenced [15]. Further study is needed to determine if 19-noraldo is also synthesized by cytochrome *P*450aldo.

18-Deoxy-19-noraldosterone is a potent antagonist of 19-noraldo [16] and 19-noraldo was converted to 18-deoxy-19-noraldo in human adrenal tissue [17]. In the present study, the 19-noraldo peak disappeared after 60 min incubation with [^{14}C]pregnenolone, suggesting that 19-noraldo may be converted to another steroid, such as 18-deoxy-19-noraldo.

REFERENCES

1. Morris D. J., Brem A. S., Saccoccio N. A., Pacholski M. and Harnik M.: Mineralocorticoid activity of 19-hydroxy-aldosterone, 19-noraldosterone, and 3β-hydroxy-Δ^5-aldosaterone: relative potencies measured in two bioassay systems. *Endocrinology* **118** (1986) 2505–2509.
2. Morris D. J., Gorsline J., Tresco P. A. and Harnik M.: The effects of 19-noraldosterone on blood pressure of adrenalectomized spontaneously hypertensive rats. *Steroids* **46** (1985) 1003–1010.
3. Rosenthal T., Shani M., Peleg E. and Harnik M.: The hypertensinogenic activity of 18-hydroxy-19-norcorticosterone in the adrenalectomized rat. *Am. J. Hypert.* 1 (1988) 49S–52S.
4. Fragman S., Harnik M., Peleg E., Zamir N. and Rosenthal T.: The hypertensinogenic activity of 18,19-dihydroxycorticosterone in adrenalectomized rats. *Am. J. Hypert.* 5 (1992) 399–401.
5. Lewicka S., Koch S., Harnik M., Cojocoaru M., Morris D. J. and Vecsei P.: Demonstration and quantitative determination of 19-noraldosterone in human urine. In *The Adrenal and Hypertension: From Cloning to Clinic* (Edited by F. Mantero, R. Takeda, B. A. Scoggins, E. G. Biglieri and J. V. Funder). Serono Symposia Publications Vol. 37, Raven Press, New York (1989) pp. 431–437.
6. Godzsa J., Vecsei P., Iwuanyanwu T. and Harnik M.: 18,19-dihydroxycorticosterone: new metabolite in human urine. *Endocrine Res.* 15 (1989) 151–157.
7. Takeda Y., Bige K., Iwuanyanwu T., Lewicka S., Vecsei P., Abdelhamid S. and Harnik M.: Urinary 18,19-dihydroxycorticosterone and 18-hydroxy-19-norcorticosterone excretion in patients with primary and secondary aldosteronism. *Steroids* **56** (1991) 566–570.
8. Takeda Y., Lewicka S., Koch S., Vecsei P., Abdelhamid S., Cojocaru M. and Harnik M.: Synthesis of 19-noraldosterone, 18-hydroxy-19-norcorticosterone and 18,19-dihydroxycorticosterone in the human aldosterone-producing adenoma. *J. Steroid Biochem. Molec. Biol.* 37 (1991) 599–604.
9. Haning S. A., Tait S. and Tait J. F.: *In vitro* effects of ACTH, angiotensins, serotonin and potassium on steroid output and conversion of corticosterone to aldosterone by isolated adrenal cells. *Endocrinology* **87** (1970) 1147–1167.
10. Takeda Y., Miyamori I., Iki K., Takeda R. and Vecsei P.: Urinary excretion of 19-noraldosterone, 18,19-dihydroxycorticosterone and 18-hydroxy-19-norcorticosterone in patients with aldosterone-producing adenoma or idiopathic hyperaldosteronism. *Acta Endocr. (Copenh.)* **126** (1992) 484–488.
11. Perrone R. D., Schwartz J. H., Bengele H. H., Dale S. L., Melby J. C. and Alexander E. A.: Mineralocorticoid activity of 19-nor-DOC and 19-OH-DOC in toad bladder. *Am. J. Physiol.* 4 (1981) E406–E409.
12. Gorosline J. and Morris D. J.: The hypertensinogenic activity of 19-nor-deoxycorticosterone in the adrenalectomized spontaneously hypertensive rats. *J. Steroid Biochem.* **23** (1985) 535–536.
13. Takeda Y., Miyamori I., Takeda R., Lewicka S. and Vecsei P.: Effect of sodium restriction on urinary excretion of 19-noraldosterone and 18,19-dihydroxycorticosterone, newly identified mineralocorticoids in man. *J. Clin. Endocr. Metab.* 74 (1992) 1195–1197.
14. Ogishima T., Mitani F. and Ishimura Y.: Isolation of aldosterone synthase cytochrome *P*-450 from zona glomerulosa mitochondria of rat adrenal cortex. *J. Biol. Chem.* **264** (1989) 10935–10938.
15. Mukai K., Imai M., Shimada H., Okada Y., Ogishima T. and Ishimura Y.: Structural differences in 5′-flanking regions of rat cytochrome *P*-450aldo and $P450_{11\beta}$ genes. *Biochem. Biophys. Res. Commun.* **180** (1991) 1187–1193.
16. Rossier B. C. : Mechanisms of action of mineralocorticoid hormones. *Endocrine Res.* **15** (1989) 203–226.
17. Takeda Y., Iki T., Miyamori I. and Takeda R.: Formation of 18-deoxy-19-noraldosterone by a human aldosterone-producing adenoma. *Steroids* **58** (1993) 282–285.