BIOSYNTHESIS OF ANDROGENS BY HOMOGENATES OF NORMAL AND ABNORMAL HUMAN ADRENAL GLANDS[1].

Shogo Ichii, Enrico Forchielli, C. E. Cassidy,
Chester B. Rosoff and Ralph I. Dorfman
Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts
New England Medical Center, Boston, Massachusetts
and
Beth Israel Hospital, Boston, Massachusetts

Received October 3, 1962

Incubation of 4-C^{14}-progesterone with a homogenate from a human normal adrenal or a virilizing adrenal adenoma yielded testosterone, androst-4-ene-3,17-dione, 17α-hydroxy-progesterone, and cortisol. On a per gram basis compared to the normal adrenal the adenoma produced 2.6 times more testosterone and 4.6 times more androst-4-ene-3,17-dione but only 0.05 times as much cortisol. The 17α-hydroxylase inhibitor SU 8000 (3- [6-chloro-3-methyl-2-indenyl] -pyridine) and SU 9055 (3-[1,2,3,4-tetrahydro-1-oxo-2 naphthyl] -pyridine inhibited the formation of cortisol, testosterone, androst-4-ene-3,17-dione and 17α-hydroxyprogesterone in the adenoma homogenate.

This is the first report of the biosynthesis of testosterone by normal human adrenal tissue.

---

1. Supported in part by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

In a previous study the biosynthetic capabilities of a virilizing adrenal adenoma was studied both *in vivo* (Burstein, S. and Dorfman, R. I., 1962) and *in vitro* (Gual et al., 1962) from the substrates, pregnenolone and cholesterol. Dehydro**epi**androsterone was formed from both steroids and evidence was obtained for a biosynthetic route in this adrenal adenoma from cholesterol to dehydroepiandrosterone not involving a C_{21} intermediate. We now report another biosynthetic study on a virilizing adrenal adenoma studied in comparison to the biosynthetic capabilities of normal adrenal tissue.

Experimental

Normal adrenal tissue was obtained from a 40-year old woman who was adrenalectomized for metastatic carcinoma of the breast and the virilizing adrenal adenoma was obtained from a 28-year old virilized woman.

Tissue in both instances was homogenized with an equal volume of 0.154 M KCl solution in an all-glass homogenizer, and the homogenates were diluted with additional KCl solution such that each 2.5 ml contained 2.0 g of tissue. For the incubation 2 gram equivalents of tissue were added to 20 ml beakers containing 0.49 μC 4-C^{14}-progesterone and was followed by the addition of 28 μMoles glucose-6-phosphate, 1.0 Kornberg unit of glucose-6-phosphate dehydrogenase, 3.5 μMoles TPN, 10 μMoles $MgCl_2$ and 30 μMoles phosphate buffer at pH 7.2. In experiments C and D the 17α-hydroxylase inhibitors SU 9055 and SU 8000 were also

added[2]. Incubations were done in a Dubnoff Metabolic Shaker in air at 37° for 2 hours.

The incubations were terminated by the addition of an equal volume of 95 percent ethanol and allowed to stand overnight in the cold. The precipitate was separated by centrifugation and washed several times with ethanol. The ethanolic extracts were defatted with petroleum ether and after concentrating to dryness 2 mg each of testosterone, androst-4-ene-3,17-dione, 17α-hydroxyprogesterone and cortisol were added to each extract as carriers. The individual extracts were subjected to a preliminary purification on silica gel columns with no attempt being made to resolve individual components. Separation and purification of each compound was accomplished by sequential paper chromatography in the ligroin, toluene, cyclohexane:benzene--propylene-glycol systems, Bush A, Bush B-3 and the benzene-methanol-water systems. Testosterone and 17α-hydroxyprogesterone were separated from each other by paper chromatography after acetylation. In this manner homogeneous zones of each compound were obtained on paper, the criteria for homogeneity being sharp correspondence between the UV absorbing zones and the radioactive peaks obtained by scanning the chromatograms. Further evidence for radiochemical purity was obtained by adding an additional 10 mg

---

2. We are indebted to Dr. J. J. Chart of Ciba Pharmaceutical Company, Summit, New Jersey, for generous supplies of SU 8000 and SU 9055.

each of the respective carriers to the eluted zones and crystalizing to constant specific activity.

Results and Discussion

In all four experiments, in terms of radioactive yield, the results are expressed as disintegrations per minute per gram of tissue. The biosynthetic products are 17α-hydroxy-progesterone, androst-4-ene-3,17-dione, testosterone and cortisol (Table 1).

Table 1

INCUBATION OF 4-C^{14}-PROGESTERONE WITH HOMOGENATES OF A NORMAL ADRENAL AND A VIRILIZING ADRENAL ADENOMA

		Disintegrations/minute/gram of tissue			
Exp.	Tissue	17α-Hydroxy--progesterone	Androst--4-ene -3,17-dione	Testo--sterone	Cortisol
A	Normal Adrenal	5035	7000	3700	133,000
B	Adrenal Adenoma	9470	32200	9800	6860
C	Adrenal Adenoma plus SU-8000	1810	257	2070	-
D	Adrenal Adenoma plus SU-9055	406	298	208	-

The data demonstrates that normal human adrenal has the potential to form testosterone from progesterone. This is the

first time that testosterone formation has been demonstrated in normal human adrenal tissue. This observation is consistent with the detection and estimation of testosterone in the plasma of ovariectomized women (Forchielli et al., 1962).

The adrenal adenoma produced 2.6 times as much testosterone per gram of tissue than the normal gland and 4.6 times more of the weaker androgen androst-4-ene-3,17-dione. Since the adenoma weighed 400 g the potential gain in the biosynthetic capacity was of the order of 100 to 200 times.

In spite of the intense increase in testosterone and androst-4-ene-3,17-dione formation cortisol production per unit weight of tissue was very low - of the order of 5% of the normal tissue. It is possible that this decrease may be due to the competitive inhibitory effect of androst-4-ene--3,17-dione on 11β-hydroxylase (3).

REFERENCES

Burstein, S. and Dorfman, R. I. Acta Endocrinol. 40, 188 (1962).

Forchielli, E., Sorcini, G., Nightingale, M., Brust, N., Dorfman, R. I., Perloff, Wm. H. and Jacobson, G. Submitted to Analytical Biochemistry.

Gual, C., Lemus, A. E., Kline, I. T., Gut, M. and Dorfman, R. I. J. Clin. Endocrinol. and Metab., Accepted, 1962.