Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 309–310. Pergamon Press. Printed in Great Britain

AROMATIZATION OF ANDROSTENEDIONE BY INDUCED POLYCYSTIC OVARIES IN THE RAT

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(*Received* 24 *April* 1975)

SUMMARY

Large polycystic ovaries were induced in 5 out of 14 hypothyroid rats treated with HCG for 20 days. The total *in vitro* conversion of [7α-^{3}H]-androstenedione to estrone by these cystic ovaries was more than 50% greater and conversion to estradiol-17β more than 40% greater than similarly treated non-cystic ovaries with an even greater increase over any of the other treatment groups. These data support a significant role for estrogen in the experimental induction of ovarian cysts and explain the capability of estrogen antagonists to inhibit cyst development.

INTRODUCTION

Polycystic ovaries, several times the normal ovarian weight in rats, are readily induced by administration of HCG to hypothyroid rats [1] and persist despite correction of the experimental conditions [2]. Estrogen has been shown to be important to the etiology of this syndrome, since the estrogen antagonist, ethamoxytriphetol (MRL-25), completely counteracts the induction of ovarian cysts while not interfering with the effect of HCG on ovarian weight [3]. However, in a study of ovarian steroidogenesis using a paper chromatographic technique for steroid analysis, aromatization of androstenedione was reported to be suppressed [4]. Since the role of estrogen secretion in ovarian cyst formation could be crucial in light of the polyendocrine involvement of this syndrome, a reinvestigation of estrogen formation using improved methods of isolation and quantification was undertaken.

EXPERIMENTAL

Twenty-nine female Sprague–Dawley rats (Carworth CFE) weighing 75–85 g were maintained for 30 days on a semi-synthetic diet of 20% casein [5] with or without 0·5% thiouracil. A portion of the animals on each diet received 10 I.U. of HCG (Antuitrin "S", Parke–Davis) daily during the last 20 days. All animals were sacrificed by cervical dislocation; the ovaries were rapidly removed, weighed, and sliced into quarters or less, depending on ovarian size. The tissues were pooled as indicated in Table 1. During quartering of the cystic ovaries the fluid was expressed and the weights used in subsequent calculations represent tissue mass unbiased by this variable.

Table 1. *In vitro* conversion of [7α-^{3}H]-androstenedione to estradiol-17β and estrone by normal, hypothyroid, gonadotrophin-stimulated and polycystic ovaries

Treatment	No. Rats	Ovarian Weight mg.	Tissue Pools		Estrone		Estradiol-17β		Ratio[c]
			No.	Weight	ng/mg[a]	Total[b]	ng/mg[a]	Total[b]	
None	5	59	1	127	1·01	59·1	1·57	91·8	0·64
Thiouracil × 30	5	42	1	90	2·06	86·4	1·70	71·5	1·21
HCG × 20[d]	5	293	2	341	0·52	151·6	1·23	359·0	0·42
Thiouracil × 30 HCG × 20 (Non-Cystic)	9	239	2	254	0·89	211·9	1·57	367·6	0·58
Thiouracil × 30 HCG × 20 (Cystic)	5	663	5	273	0·96	526·8	1·06	591·1	0·89

[a] Concentration of labeled estrogen as ng/mg of tissue minus cyst fluid.
[b] Ng per pair of ovaries.
[c] Ratio of estrone to estradiol-17β.
[d] HCG administered at dose of 10 I.U. sc daily for the last 20 days of the experimental period.

The ovarian tissue fragments were incubated for 4 h in vials containing 2 ml of Krebs-Ringer bicarbonate buffer with 1 μg and 2 μCi of [7α-^{3}H]-androstenedione (Amersham–Searle, 3·1 Ci/mM) and quickly frozen. After thawing, 0·2 ml of steroid carrier solution was added containing 0·2 mg each of estrone and estradiol-17β, 0·1 mg each of androstenedione and testosterone, and 0·05 μCi of [4-^{14}C]-estradiol-17β (Nuclear Chicago, 31·8 mCi/mmol). The tissue was homogenized in its medium and extracted according to the procedure of Stitch *et al.*[6] into isopropyl ether. Phenolic steroids were extracted 3 times with 5 ml of 1N NaOH; the pooled extracts were washed with isopropyl ether, acidified with 5 N H_2SO_4, and extracted 3 times with 5 ml of benzene.

The benzene extracts were combined, reduced to 1·0 ml and applied to a 1 cm. column packed to a height of 16 cm. with 5 g of Celite 535 [7] coated with 4·5 ml of 0·8 N NaOH saturated with benzene [8]. The column was eluted at 10 C with benzene saturated with 0·8 N NaOH in 2 ml fractions until about 50 ml were collected. Dichloroethane/benzene (3:1 v/v) was used to collect an additional 75 ml. Peaks of radioactivity were identified by differential counting of 0·1 ml aliquots of alternate fractions in 5 ml of Bray's solution in a Packard Tri-Carb scintillation spectrometer. Fractions corresponding to estrone and to estradiol-17β [6] were pooled, reduced to a constant volume, and differentially counted to establish content of ^{3}H and the ^{14}C carrier estradiol-17β; the counts of the individual fractions were added to the counts of the pools. Elution sequence was taken as the criterion of identity of the phenolic steroids [6] and no further purification was attempted. Overall recovery of [^{14}C]-estradiol-17β from the entire procedure was quite uniform averaging 50% ($\pm$1·4).

RESULTS AND DISCUSSION

Thirty days of goitrogen feeding suppressed ovarian weight and maturation (Table 1). Stimulation of euthyroid animals with HCG for 20 days induced a 5-fold increase in ovarian size with predominantly lutein tissue development. In hypothyroid animals, HCG produced ovaries weighing an average of 391 mg for the 14 animals in the study, and a high incidence of follicular cysts occurred with consequent maximum ovarian weight of 884 mg in one animal.

Ovaries from hypothyroid rats differed little from controls in their ability to convert androstenedione to estradiol-like phenolic steroids; whereas synthesis of estrone-like material was somewhat higher, no confidence can be placed in these differences since they are not based on replicate samples. Furthermore, these data would be at variance with reports that hypothyroidism reduces aromatization of androgens [9] as well as steroidogenesis in general [4, 10].

The ovarian hypertrophy induced by HCG in euthyroid animals was characterized by a total "estrone" production more than 3 times that of the controls and a 4-fold increase in the synthesis of steroids corresponding to the more potent estradiol-17β (Table 1). In hypothyroid rats given HCG for 20 days, 9 of the 14 animals showed little difference in morphology or estrogen formation in comparison to euthyroid HCG-treated animals. In the 5 animals with ovarian cysts (gonadal weights ranging from 479 to 884 mg) aromatization of androgen precursor to estrone was higher and to estradiol-17β lower than HCG-treated euthyroid ovaries on the basis of concentration. The ratio of the estrone fraction to the estradiol-17β fraction was higher than control animals and approached the values found in uninjected hypothyroid rats rather than the depressed ratio characteristic of HCG-treated ovaries in the euthyroid animal. Nevertheless, the total production of estradiol-17β by each pair of cystic ovaries was 40% higher than either the ovaries of euthyroid, HCG-treated rats or the non-cystic ovaries of hypothyroid, HCG-treated rats. Steroids in the estrone fraction were formed by cystic ovaries at more than twice the rate of either HCG-treated group.

The total production of phenolic steroids from androgen precursor by cystic ovaries is clearly enhanced since the tissue mass is so much greater than in non-cystic ovaries. An increased release of estrogenic hormone may be presumed but should be measured specifically. However, the earlier report [3] that inhibition of estrogen action prevents ovarian cyst formation strongly suggests that an elevation in estrogen production and release is a significant factor in the induction of polycystic ovaries in the rat.

REFERENCES

1. Leathem J. H.: *Ciba Found. Colloquia on Endocrinol.* **12** (1958) 173–187.
2. Leathem J. H.: *Anat. Rec.* **131** (1958) 487–500.
3. Leathem J. H. and Adams W. C.: *Proc. Soc. exp. Biol. Med.* **113** (1963) 240–242.
4. Callard G. V. and Leathem J. H.: *Proc. Soc. exp. Biol. Med.* **118** (1965) 996–999.
5. Leathem J. H. and Wolf R. C.: *Proc. Soc. exp. Biol. Med.* **86** (1954) 724–725.
6. Stitch S. R., Oakey R. E. and Eccles S. S.: *Biochem. J.* **88** (1963) 70–76.
7. Preedy J. R. K. and Aitken E. H.: *J. biol. Chem.* **236** (1961) 1300–1311.
8. Bauld W. S.: *Biochem. J.* **59** (1955) 294–300.
9. Lehman W. C., Lehmann V. and Brandau H. *Endokrinologie* **57** (1970) 115–124.
10. Shapiro B. H. and Leathem J. H.: *Proc. Pa. Acad. Sci.* **42** (1968) 227–229.