Effect of Some Contraceptive Steroids on Pituitary Growth Hormone Content in Female Rats[1] (34688)

FRANK T. Y. LIU AND HSIEN S. LIN

Department of Physiology, University of Missouri-Kansas City, School of Dentistry, Kansas City, Missouri 64110

Prolonged administration of relatively large doses of ethynylestradiol 3-methyl ether (mestranol) and/or 17 α-ethynyl-17β-hydroxy-5(10)-estrin-3-one(norethynodrel), which are widely used as oral contraceptives, was found to cause a significant retardation of body and organ growth in female rats (1, 2). This report describes the effect of these synthetic contraceptive steroids on the growth hormone (GH) content of the anterior pituitary in some of these treated rats.

Materials and Methods. The donor rats of this experiment were the same groups of rats treated with higher doses of steroid(s) and the controls as those in Expt. 3 which was reported previously (2). In short, they were fed *ad libitum* and beginning on day 23 those rats in the experimental groups received, per 100 g of body wt, sc injections of 50 μg of mestranol[2] 1250 μg of norethynodrel[2] or their combined treatment on Mondays, Wednesdays, and Fridays for 10 weeks, controls, received vehicle alone. At autopsy, the anterior pituitary glands (AP) were removed and weighed immediately on a torsion balance, they were then pooled and frozen for future assay of growth hormone content.

The pooled AP of each group of rats were homogenized, prior to GH assay, in a ground glass homogenizer, and diluted with physiological saline so that each 2 ml of homogenate contained ¼ AP, ½ AP, or 1 AP. The assay rats (recipients)[3] were hypophysectomized (hypox) on day 27 and were fed as instructed. Beginning on day 30 they were given daily ip injections of 0.5 ml of one of the AP homogenates for 4 days. Thus, each recipient received a total of ¼ AP, ½ AP, or 1 AP during a 4-day period. The hypox controls were similarly treated with physiological saline. The tibial epiphyseal cartilage of the recipients and hypox controls was prepared and its width was measured by a standard procedure (3) and as that described previously (4). Mean final body weight and body weight gain, as well as the absolute and relative (AP wt in mg/100 g of body wt) AP weight, of each treated group of donors were compared with the controls by Student's *t* test. Similarly the mean epiphyseal cartilage widths of the recipients receiving different amounts of AP obtained from the three steroid-treated groups of donors were also compared with those of recipients receiving an appropriate amount of AP obtained from control donors. Comparisons were also made between the mean epiphyseal cartilage widths of hypox controls and those of the recipient given different amounts of AP obtained from steroid-treated donors. The differences between the mean parameters were considered as statistically significant when p values were less than 0.05.

Results. Table I shows that the mean final body weight and body weight gain, as well as the absolute weight of AP, of all steroid-treated groups of rats decreased significantly as compared to the controls. The body sizes of all steroid-treated groups of rats also were

[1] This investigation was supported in part by NIH Research Grant DE-01621 at the University of Pittsburgh and by the USPHS General Research Support Grant FR-05323 of the University of Missouri-Kansas City, School of Dentistry.

[2] G. D. Searle and Co., Chicago, Illinois.

[3] Hypophysectomized by the Hormone Assay Laboratory, Chicago, Illinois.

TABLE I. Effect of Norethynodrel, Mestranol, or Their Combination on Body and Anterior Pituitary Weight in Female Rats.

Treatment[a]	Vehicle (12 rats)	Norethynodrel, 1250 μg (18 rats)	Mestranol, 50 μg (16 rats)	Norethynodrel, 1250 μg + mestranol, 50 μg (23 rats)
Body wt (g)				
Final	244 ± 3.5[b]	151 ± 2.6[c]	157 ± 2.6[c]	146 ± 2.1[c]
Gain	199 ± 3.2	106 ± 2.7[c]	112 ± 2.9[c]	102 ± 2.1[c]
Anterior pituitary gland				
(mg)	10.9 ± 0.17	5.5 ± 0.17[c]	6.4 ± 0.22[c]	4.9 ± 0.14[c]
(mg/100 g of body wt)	4.5 ± 0.12	3.7 ± 0.10[c]	4.1 ± 0.17	3.7 ± 0.10[c]

[a] Subcutaneous injections on Mondays, Wednesdays, and Fridays for 10 weeks.
[b] Mean ± SE.
[c] $p < 0.001$, compared to appropriate controls.

markedly smaller than the controls. Although the mean relative wet AP weight of norethynodrel- or norethynodrel plus mestranol-treated rats decreased significantly, the mean relative AP weight of mestranol-treated rats decreased slightly as compared to the controls.

As judged by the tibial epiphyseal cartilage width of the hypox recipients, the GH content of AP of all three treated groups of donors decreased significantly in comparison with that of the AP of control donors (Table II). Furthermore, the degree of these differences of cartilage widths increased with increase in the amount of donor's AP given. Nevertheless, the AP of the donors of any of the steroid-treated groups contained a moderate amount of GH. This was judged by a

TABLE II. Effect of Norethynodrel, Mestranol, or Their Combination in Pituitary Growth Hormone Content in Female Rats.

Treatment received by donor rats	Hypophysectomized assay rats			
	Amount of donor's AP[a] /rat/4 days	No. of rats	Epiphyseal cartilage width μ	Epiphyseal cartilage width p value
Vehicle	¼	7	234.1 ± 4.3[b]	—
Norethynodrel	¼	10	216.3 ± 4.1	<0.010
Mestranol	¼	8	209.5 ± 7.1	<0.025
Norethynodrel + mestranol	¼	8	213.0 ± 6.8	<0.025
Vehicle	½	7	265.1 ± 3.7	—
Norethynodrel	½	9	233.7 ± 5.7	<0.001
Mestranol	½	9	242.4 ± 3.9	<0.001
Norethynodrel + mestranol	½	10	229.6 ± 6.0	<0.001
Vehicle	1	6	309.3 ± 4.9	—
Norethynodrel	1	9	259.8 ±10.0	<0.001
Mestranol	1	8	283.4 ± 6.7	<0.001
Norethynodrel + mestranol	1	10	248.4 ± 5.6	<0.001
—	None	14	161.2 ± 6.0	<0.001[c]

[a] Anterior pituitary from the same groups of rats as shown in Table I.
[b] Mean ± SE.
[c] Compared with any cartilage width of assay rats received any amount of AP obtained from all steroid-treated donors.

significant increase ($p < 0.001$) in mean epiphyseal cartilage widths of all groups of recipients which received any given amount of AP obtained from all steroid-treated groups of donors as compared to that of hypox controls.

Discussion. Under the present experimental conditions mestranol, norethynodrel, or their combined treatment caused a significant decrease in GH content in rat AP as compared to the controls. These findings agree with the report that a combined oral administration of mestranol and norethynodrel in immature rats caused a significant decrease in tibial length as compared to that of pair-fed controls (5). Since the wet weight of AP of treated rats also decreased, the GH concentration of AP may have not been affected by the treatment. The decreased relative wet AP weight in steroid-treated rats indicated that the AP growth was retarded to a greater extent than that of general body growth.

The action mechanism of these steroid-induced decreases in GH content or the wet weight of rat AP is not known. As judged by the decrease in body weight gain and body size of steroid-treated rats, it appeared that there was probably no elevated plasma GH concentration, which also would indicate that the decreased GH content of AP in these treated rats was probably not due to a persistently increased release of GH from the AP. Therefore, the steroid treatment might have inhibited, directly or indirectly through the hypothalamus, the pituitary GH synthesis and release or the decreased GH content of the treated rat AP was simply due to the significant decrease in gland weight. Yet, it has been reported that in women receiving combination-type or sequential-type oral contraceptive steroids, there was an elevation of plasma GH level, especially a temporarily marked elevation of plasma GH level in response to insulin-induced hypoglycemia (6, 7). Thus, the administered contraceptive steroids might have stimulated the GH release from rat AP as it does in humans but these steroids might have exerted, concurrently, a biologically antagonistic action on GH at the level of peripheral tissues as was suggested in the report of estrogen administration in girls (8). Therefore, it would be of interest to study whether or not there are any changes in plasma GH level in steroid-treated rats or whether administration of large doses of GH would prevent the steroid-induced retardation in body growth. Since a complete starvation from 2 to 7 days was reported to decrease GH content of rat AP (9, 10) and it was observed that these steroid-treated rats consumed slightly less food, the decreased pituitary GH content might have resulted from malnutrition. However, in pair-fed experiment the steroid-treated immature rats gained less body weight and had significantly shorter tibial length as compared to the controls (5). Therefore, the slightly decreased food consumption by the steroid-treated rats in the present experiment was probably not an important factor in causing decreased GH content of the AP.

It is known that mestranol exerts a potent and norethynodrel has a slight estrogenic activity (11). It is possible that the estrogenic activity of mestranol and norethynodrel was responsible for the inhibition of GH synthesis by rat AP. This hypothesis is supported by the findings that a prolonged administration of large doses of naturally occurring and synthetic estrogens (estradiol, estrone, estriol, or ethynylestradiol) caused a significant retardation of body growth in rats and in humans (2, 12–16); whereas, administration of large doses of synthetic or naturally occurring progestogenic or androgenic steroids (medroxyprogesterone acetate, progesterone, and testosterone) stimulated the body growth or caused an increase in pituitary GH content and concentration in rats (14, 17, 18).

Summary. Weanling female Sprague-Dawley strain rats were given sc injections of 50 μg of mestranol, 1250 μg of norethynodrel or their combination three times weekly for 10 weeks, controls received vehicle. Anterior pituitary glands (AP) of each of the steroid-treated groups of donors were pooled for assay of GH content by measuring tibial epiphyseal cartilage width of hypophysectomized recipients. Recipients received four ip injections of AP homogenates containing a total of ¼ AP, ½ AP, or 1 AP; control recip-

ients received saline solution alone. As judged by tibial cartilage width of the recipients, the GH content of the AP of any of the three steroid-treated groups of donors decreased significantly as compared to that of the AP of the control donors.

The authors wish to thank Dr. Hamilton B. G. Robinson, Dean of School of Dentistry, University of Missouri-Kansas City for his encouragement in preparing this manuscript and G. D. Searle and Company, Chicago, Illinois for the generous supply of norethynodrel and mestranol.

1. Liu, F. T. Y., Lofgren, R., and Hoots, I., J. Dent. Res. **45**, 1236 (1966).

2. Liu, F. T. Y., J. Dent. Res. **47**, 1047 (1968).

3. "Methods in Hormone Research." (Dorfman, R. I., ed.), Vol. 3, p. 684. Academic Press, New York (1962).

4. Liu, F. T. Y., and Lin, H. S., J. Dent. Res. **48**, 559 (1969).

5. Yang, M. G., Sanger, V. L., and Mickelsen, O., Proc. Soc. Exp. Biol. Med. **130**, 1146 (1969).

6. Spellacy, W. N., Buhl, W. C., and Bendel, R. P., Obstet. Gynecol. **33**, 506 (1969).

7. Spellacy, W. N., Buhl, W. C., and Bendel, R. P., Int. J. Fert. **14**, 51 (1969).

8. Frantz, A. G., and Rabkin, M. T., J. Clin. Endocrinol. **25**, 1470 (1965).

9. Friedman, R. C., and Reichlin, S., Endocrinology **76**, 787 (1965).

10. Dickerman, E., Negro-Vilar, A., and Meites, J., Endocrinology **84**, 814 (1969).

11. Drill, V. A., "Oral Contraceptives," pp. 9, 11. McGraw-Hill, New York (1966).

12. Liu, F. T. Y., Proc. Soc. Exp. Biol. Med. **124**, 591 (1967).

13. Liu, F. T. Y., J. Dent. Res. **46**, 471 (1967).

14. Liu, F. T. Y., and Lin, H. S., J. Dent. Res. **48**, 477 (1969).

15. Liu, F. T. Y., Lin, H. S., and Zullo, T. G., J. Dent. Res. **48**, 485 (1969).

16. Greenblatt, R. B., McDonough, P. G., and Mahesh, V. B., J. Clin. Endocrinol. **26**, 1185 (1966).

17. Liu, F. T. Y., and Lin, H. S., J. Dent. Res. **48**, 943 (1969).

18. Daughaday, W. H., Peake, G. T., Birge, C. A., and Mariz, I. K., *in* "Proceedings of the First International Symposium on Growth Hormone" (A. Pecile and E. E. Müller, eds.), p. 243. Excerpta Medica, Amsterdam (1968).

Received Dec. 3, 1969. P.S.E.B.M., 1970, Vol. 133.