PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 66–69 (1979)

Effects on Gonadotropin Secretion of 5-α-Pregnane-3α, 20α-Diol, a Physiological Progesterone Derivative[1] (40491)

MARIAROSA ZANISI AND LUCIANO MARTINI

Department of Endocrinology, University of Milan, 20129 Milano, Italy

The rat ovary secretes, in addition to progesterone, a series of other related steroids. Among these are prominent 20α-hydroxy-pregn-4-en-3one (20α-progesterone, 20α-P) (1–6), and its 5α-reduced metabolite 5α-pregnane-3α, 20α-diol (5α-diol) (6–8) (see Fig. 1). The anterior pituitary, the hypothalamus and other nervous structures of the rat (9–12) are able to convert progesterone into 5α-pregnane-3,20-dione (5α-DHP) and into 5α-pregnane-3α-ol-20-one (3α-OL) (see Fig. 1). More recently, it has been demonstrated that the neuroendocrine tissues are also able to convert progesterone into 20α-P, and subsequently into 5α-pregnan-20alpha-ol-3-one (20α-DHP), and 5α-diol (13–15). 20α-P has been shown to be an even better substrate for the 5α-reductase of the rat anterior pituitary (16).

Progesterone is known to induce ovulation and to facilitate LH release in the female rat (17), and is believed to play a physiological role in the neuroendocrine processes leading to the preovulatory gonadotropic surge in this species (18–22). The participation of 20alpha-P in these processess is suggested by the fact that the secretion of this steroid from the ovary increases at proestrus, before the initiation of the LH preovulatory surge (1–5, 23). Moreover, it has been shown that 20alpha-P is able to release LH and FSH in ovariectomized estrogen-primed rats (18, 19). Finally, Hilliard and coworkers (24) have suggested that 20α-P may amplify the amounts of LH released after mating in rabbits. However, divergent data have also appeared. For instance, Fink and Henderson (20) were unable to obtain gonadotropin release in estrogen-primed female rats after administering 20α-P, and Goodman and Neill (25) could not duplicate the results of Hilliard *et al.* (24) in does.

In recent studies, it has been shown that the physiological 5α-reduced metabolites of progesterone 5α-DHP and 3α-OL, are able to provoke a large release of LH and FSH when given to castrated-estrogen-primed female rats (19–21, 26). The present investigation has been performed: (a) to gain additional information on the effects of 20α-P on the control of gonadotropin release; and (b) to determine whether the 5α-reduced metabolite of 20α-P, 5alpha-DIOL might exert any effect on gonadotropin secretion. To the authors' knowledge, the activity of this steroid on gonadotropin release has never been investigated.

Materials and methods. Animals. All experiments have been performed in adult female rats of the Sprague-Dawley strain. Animals were caged in rooms with controlled temperature and humidity (lights on from 6.30 to 20.30); they were fed with a standard pellet diet and water was allowed *ad libitum*.

In order to analyse whether the two steroids might exert a stimulatory effect on gonadotropin release, an approach similar to that of Swerdloff *et al.* (18) was adopted. These authors have shown that a gonadotropin surge is induced, in ovariectomized rats, by the daily administration of small doses of ethinyl estradiol (EE) (0.4 μg/day) followed by the administration of progesterone or of some progesterone derivatives. Animals were ovariectomized 15 days before initiating the experiment, when weighing 150 ± 10 g. Subsequently they were subdivided into five groups of four to five animals each. The first group (controls) was injected daily with soya oil; the remaining four groups received daily subcutaneous treatments with 0.4 μg/rat of EE. All injections were performed at 8.30. On the fifth day, the five groups of rats received at 8.30 the following subcutaneous treatments: Group 1: soya oil; Group 2: 0.4 μg EE; Group 3: 0.4 μg EE + 100 μg progesterone;

[1] This work was supported by a grant from the Italian National Council of Research (CNR) through the project "Biology of Reproduction" and by a grant from the Ford Foundation, New York, NY.

0037-9727/79/050066-04$01.00/0

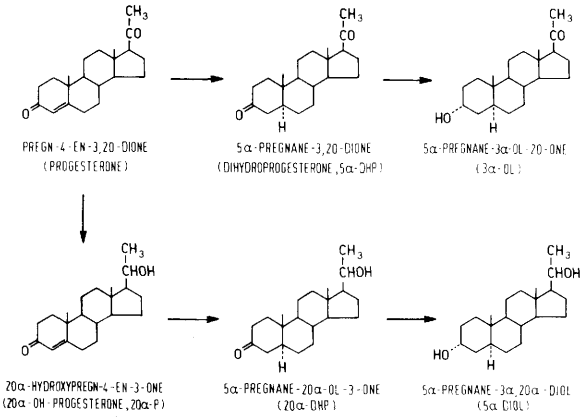

FIG. 1. Pathways involved in progesterone and 20α-hydroxyprogesterone metabolism.

Group 4: 0.4 μg EE + 100 μg 20α-P; Group 5: 0.4 μg EE + 100 μg 5alpha-DIOL. All steroids were dissolved in soya oil. The animals were sacrificed by decapitation the same day at 17.30 and blood was collected for the evaluation of serum levels of LH and FSH.

In order to study the possible inhibitory effect of the various progesterone derivatives on gonadotropin release, adult female rats (initial weight 150 ± 10 g) were ovariectomized 21 days prior to the beginning of the study and divided into four groups of four to five animals each. On day 21 after castration, the animals were injected subcutaneously at 8.30 in the following fashion: Group 1: soya oil; Group 2: 2 mg Progesterone; Group 3: 2 mg 20α-P; Group 4: 2 mg 5α-DIOL. The animals were killed 24 hr later by decapitation and blood was collected for the evaluation of serum levels of LH and FSH.

Serum LH was determined by the procedure described by Niswender *et al.* (27) and values have been expressed in terms of NIH-LH-S17. FSH was measured using the NIAMDD-rat-FSH-radioimmunoassay kit, according to the method of Daane and Parlow (28). Values have been expressed in terms of NIAMDD-rat-FSH-RP1. Statistical analysis of the data has been performed using the "t" test after one way analysis of variance (29).

Results. It is clear from Table I that long-term ovariectomized rats have elevated serum levels of both LH and FSH and that these can be significantly decreased by the 5-day administration of small doses of estrogen. In line with previous observations, progesterone (18–21, 26) and 20α-P (18, 19) are able to exert a strong stimulatory effect on the release of LH in ovariectomized estrogen-primed animals. A stimulatory effect of comparable magnitude is also observed following the administration of 5α-DIOL. Progesterone, when given to estrogen-primed animals, significantly increased serum levels of FSH, even if its potency was not as great as that observed in the case of LH. This is similar to a previous report of this and other laboratories (19, 26) but conflicts with data of Swerdloff *et al.* (18). In the present experiment 20α-P slightly and

TABLE I. EFFECT OF S.C. INJECTION OF 100 μg/RAT OF PROGESTERONE (*P*), 20α-hydroxy-pregn-4-en-3one (20alpha-P) AND 5α-pregnane-3α,20α-DIOL (5α-DIOL) ON SERUM LEVELS OF LH AND FSH OF CASTRATED FEMALE RATS PRETREATED FOR 5 DAYS WITH ETHINYL ESTRADIOL (EE, 0.4 μg/RAT/DAY) AND SACRIFICED 8 hr AFTER PROGESTAGEN ADMINISTRATION.

Group	Treatment[a]	ng LH/ml (NIH-LH-S17)[b]	ng FSH/ml (NIAMDD-rat FSH-RP1)[b]
1.	Oil + oil	(4) 21.61 0.57	2620 ±273
2.	EE + EE	(4) 0.325 ±0.09[c]	1455 ±124[c]
3.	EE + P	(5) 12.042 ±0.62[d]	1882 ±115[d]
4.	EE + 20α-P	(5) 11.872 ±0.31[d]	1648 ±77
5.	EE + 5alpha-DIOL	(5) 10.712 ±0.24[d]	922 ±51[d]

[a] Numbers of animals in parentheses.
[b] Values are means ± SE.
[c] 2 *vs* 1: $P < 0.005$.
[d] *vs* 2: $P < 0.005$.

not significantly increased serum levels of FSH, while 5α-DIOL brought about a significant reduction of FSH release.

Progesterone, when given to castrated animals, in the absence of estrogen priming, exerted a slight inhibitory effect on the release of both LH and FSH (Table II). 5α-DIOL did not exert any effect on LH and FSH secretion in the absence of estrogen priming. Some inhibition of LH secretion was observed following the administration of 20α-P, but this steroid did not modify FSH secretion.

Discussion. The present data confirm that, in ovariectomized estrogen-primed rats 20α-P may exert a strong activatory effect on LH secretion (18, 19), which is quantitatively similar to that of progesterone. Unlike progesterone, 20α-P stimulated FSH release only slightly under the experimental conditions adopted. The 5alpha-reduced derivative of 20α-P, 5α-DIOL exerted a strong stimulatory action on LH release, but inhibited FSH secretion. This last result suggests that also this 5α-reduced steroid might participate in the physiological control of gonadotropin secretion, either by reaching the CNS-pituitary complex via the systemic circulation (since it is a major component of the progestin quota secreted by the ovary) (6–8), or by being formed directly in the brain and in the anterior pituitary from 20α-P or from progesterone (see introduction) (13–16). However, this hypothesis needs to be confirmed by additional studies, since only one dose has been tested in the present experiments.

The progesteronelike effects of 5α-DIOL cannot be attributed to the back conversion of this metabolite to 20α-P or to progesterone because the reduction of the double bond in progesterone is irreversible (12, 30), and because 5α-DIOL exerts effects on LH and FSH, which are different from those of 20α-P and of progesterone. The differential response of the two gonadotropins to the administration of 5α-DIOL (stimulation of LH, inhibition of FSH) supports the concept that LH and FSH secretion may be regulated in an independent fashion (31, 32).

The present data indicate that progesterone and 20α-P may inhibit LH release in the absence of exogenous estrogens. Moreover, progesterone may also affect FSH secretion. On the contrary, the 5α-reduced derivative studied does not exert any suppressive effect on either gonadotropins in animals without estrogen pretreatment. These data may suggest that the process of 5α-reduction plays a role in the induction of the "positive" but not of the "negative" feedback activity of progestogens. A similar conclusion has been reached by Nuti and Karavolas (21).

Summary. 5α-pregnane-3α,20α-DIOL (5α-DIOL), a steroid which is secreted by the rat ovary and which may be formed from progesterone and 20α-hydroxyprogesterone in the brain and in the anterior pituitary, stimulates LH release while inhibiting FSH secretion in ovariectomized estrogen-primed rats. The steroid does not influence gonadotropin secretion when given to castrated female rats in the absence of exogenous estrogens. These data suggest that the process of 5α-reduction plays a role in the induction of the "positive" but not of the "negative" feedback activity of progestogens.

TABLE II. EFFECT OF S.C. INJECTION OF 2 mg/RAT OF PROGESTERONE (P), 20α-hydroxy-pregn-4-en-3one (20α-P) and 5α-pregnane-3alpha, 20α-DIOL (5α-DIOL) ON SERUM LEVELS OF LH AND FSH OF CASTRATED FEMALE RATS SACRIFICED 24 HR AFTER PROGESTAGEN ADMINISTRATION.

Group	Treatment[a]	ngLH/ml (NIH-LH-S17)[b]	ngFSH/ml (NIAMDD-rat-FSH-RP1)[b]
1.	Oil	(4) 21.99 ± 0.49	2759 ± 22
2.	P	(5) 19.244 ± 0.01[c]	2135 ± 138[d]
3.	20α-P	(5) 19.058 ± 0.81[c]	2458 ± 178
4.	5α-DIOL	(5) 20.952 ± 0.51	2453 ± 187

[a] Number of animals in parentheses.
[b] Values are means ± SE.
[c] 2 *vs* 1: $P < 0.005$.
[d] 2 *vs* 1: $P < 0.05$.

Thanks are due to Drs. A. R. Midgley, Jr., L. E. Reichert and G. D. Niswender and to the National Institute of Arthritis and Metabolic Digestive Diseases, Rat Pituitary Hormone Distribution Program for the gift of the radioimmunoassay materials.

1. Feder, H. H., Resko, J. A., and Goy, R. W., J. Endocrinol. **41**, 563 (1968).
2. Hashimoto, I., Henrichs, D. M., Anderson, L. L., and Melampy, R. M., Endocrinology **82**, 333 (1968).
3. Goldman, B. D., Kamberi, I. A., Siiteri, P. K., and Porter, J. C., Endocrinology **85** 1137 (1969).
4. Barraclough, C. H., Collu, R., Massa, R., and Martini, L., Endocrinology **88**, 437 (1971).

5. Rubin, B. L., and Chase, H. S., Jr., Steroids **18,** 59 (1971).
6. Ichikawa, S., Sawada, T., Nakamura, Y., and Morioka, H., Endocrinology **94,** 1615 (1974).
7. Holzbauer, M., Brit. J. Pharmacology **43,** 560 (1971).
8. Holzbauer, M., J. Steroid Biochem. **6,** 1307 (1975).
9. Massa, R., Stupnicka, E., and Martini, L., Program 4th Intern. Congr. Endocrinology, p. 118 (1972).
10. Karavolas, H. J., and Nuti, K. M., *in* "Subcellular Mechanisms in Reproductive Neuroendocrinology" (F. Naftolin, K. J. Ryan, and I. J. Davies, eds.), p. 305, Elsevier, Amsterdam (1976).
11. Stupnicka, E., Massa, R., Zanisi, M., and Martini, L., *in*: "Clinical Reproductive Neuroendocrinology" (P. O. Hubinont, M. L'Hermite, and C. Robyn, eds.), p. 88, Karger, Basel (1977).
12. Karavolas, H. J., Hodges, D., and O'Brien, D., Endocrinology **98,** 164 (1976).
13. Tabei, T., Haga, H., Heinrichs, W. L., and Herrmann, W. L., Steroids **23,** 651 (1974).
14. Nowak, F. V., and Karavolas, H. J., Steroids **24,** 351 (1974).
15. Celotti, F., Massa, R., and Martini, L., *in* "Endocrinology: Metabolic Basis of Clinical Practice" (L. J. de Groot, ed.), p. 41, Grune and Stratton, New York (1978).
16. Cheng, Y. J., and Karavolas, H. J., Steroids **26,** 57 (1975).
17. Everett, J. W., Physiol. Rev. **44,** 373 (1964).
18. Swerdloff, R. S., Jacobs, H. S., and Odell, W. D., Endocrinology **90,** 1529 (1972).
19. Brown-Grant, K., J. Endocrinol. **62,** 319 (1974).
20. Fink, G., and Henderson, S. R., J. Endocrinol. **73,** 157 (1977).
21. Nuti, K. M., and Karavolas, H. J., Endocrinology **100,** 777 (1977).
22. Piva, F., Motta, M., and Martini, L., *in* "Endocrinology: Metabolic Basis of Clinical Practice" (L. J. de Groot, ed.), p. 21, Grune and Stratton, New York (1978).
23. Piacsek, B. E., Schneider, T. C., and Gay, V. L., Endocrinology **89,** 39 (1971).
24. Hilliard, J., Penardi, R., and Sawyer, C. H., Endocrinology **80,** 901 (1967).
25. Goodman, A. L., and Neill, J., Endocrinology **99,** 852 (1976).
26. Zanisi, M., and Martini, L., J. Steroid Biochem. **6,** 1021 (1975).
27. Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., and Reichert, L.E., Jr., Proc. Soc. Exp. Biol. Med. **128,** 807 (1968).
28. Daane, T. A., and Parlow, A. F., Endocrinology **88,** 653 (1971).
29. Ermitage, P., *in* "Statistical Methods in Medical Research" Blackwell Scientific Publication (1971).
30. Wiest, W. G., *in* "The Sex Steroids" (K. W. McKerns, ed.), p. 295, Appleton-Century-Crofts, New York (1972).
31. Zanisi, M., and Martini, L., Acta Endocrinol. **78,** 683 (1975).
32. Zanisi, M., and Martini, L., Acta Endocrinol. **78,** 689 (1975).

Received September 18, 1978. P.S.E.B.M. 1979, Vol. 161.