*Journal of Steroid Biochemistry*, 1975, Vol. 6, pp. 1021–1023. Pergamon Press. Printed in Great Britain.

# EFFECTS OF PROGESTERONE METABOLITES ON GONADOTROPHIN SECRETION

M. ZANISI and L. MARTINI

Department of Endocrinology, University of Milan, Milan, Italy

It has recently been reported that in several of its target structures (uterus, anterior pituitary, hypothalamus, etc.) progesterone is metabolized into the following 5α-reduced metabolites: 5α-pregnane-3,20-dione (dihydroprogesterone, DHP) and 5α-pregnan-3α-ol-20-one (3α-ol) [1–4]. The hypothesis has been put forward that the formation of these compounds represents an essential step for progesterone to exert its biological effects [1–4]. Such a hypothesis is similar to the one proposed for explaining the mode of action of testosterone. It is generally accepted that testosterone must be locally converted into 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT), 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol), in order to fully express its multiple activities on androgen-sensitive structures [5–8].

Progesterone (P) is known to influence the secretion of anterior pituitary gonadotrophins. Depending on the circumstances, P may exert either inhibitory (negative feedback effect) or stimulatory (positive feedback or facilitatory effect) actions [9, 10]. The experiments here to be described have been planned in order to validate the hypothesis that DHP and 3α-ol might represent the mediators of P actions in the structures where the steroid exerts its feedback effects on LH and FSH secretion. The activities of DHP and 3α-ol have been evaluated in the rat, using two different experimental approaches, designed to test respectively the inhibitory and the facilitatory activities of these steroids on gonadotrophin secretion. 5α-Pregnan-3β-ol-20-one (3β-ol) (the 3β epimer of 3α-ol) was also included in this study. Normally P-sensitive structures convert P into this metabolite to a very limited extent [3, 11]. However, this steroid may play some physiological role since it is produced in the ovaries and secreted into the ovarian vein [12].

In order to analyse whether DHP, 3α-ol and 3β-ol are able to stimulate gonadotrophin release, these steroids have been administered to castrated oestrogen-pretreated female rats. It is a well documented phenomenon that P and several physiological or synthetic progestogens (20α-hydroxyprogesterone, 17α-hydroxyprogesterone, etc.) exert a positive feedback effect on LH and/or FSH secretion in animals so prepared [13–17]. In order to test their inhibitory activity on gonadotrophin secretion, DHP, 3α-ol and 3β-ol were administered in rather large amounts to castrated female rats without any oestrogen pretreatment. Details on the procedures adopted are given in the section "Materials and Methods".

## 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 a.m. to 8.30 p.m.), and were fed with a standard pellet diet; water was given *ad libitum*. Animals were submitted to castration when weighing 150 ± 10 g.

### *Positive feedback effect*

An approach similar to that adopted by Swerdloff *et al.*[15] was used for this type of experiment. Adult female rats were ovariectomized (regardless of the phase of the oestrous cycle) 15 days prior to the beginning of the study and divided into six groups. Beginning on the 15th day after castration, five groups were submitted to treatment with ethinyl estradiol (EE). EE was dissolved in sesame oil, and injected subcutaneously for four consecutive days at 8.30 a.m. in the daily dose of 0·4 μg/rat. The sixth group was similarly injected for 4 days with sesame oil. Treatment with these doses of EE has been previously shown to bring back to precastration levels the elevated gonadotrophin titres of castrated animals [15, 18]. On the fifth day, the six groups of rats received the following subcutaneous treatments: Group 1: 0·4 μg EE; Group 2: 0·4 μg EE + 100 μg P; Group 3: 0·4 μg EE + 100 μg DHP; Group 4: 0·4 μg EE + 100 μg 3α-ol; Group 5: 0·4 μg EE + 100 μg 3β-ol; Group 6: sesame oil. The animals were sacrificed by decapitation the same day at 5.30 p.m. Blood was collected in order to evaluate serum levels of LH and FSH.

### *Negative feedback effect*

For these experiments, adult female rats were ovariectomized (regardless of the phase of the oestrous cycle) 21 days prior to the beginning of the study, and divided into 5 groups. On day 21 after castration, the animals were injected subcutaneously at 8.30 a.m. in the following fashion: Group 1: 2 mg P; Group 2: 2 mg DHP; Group 3: 2 mg 3α-ol; Group 4: 2 mg 3β-ol; Group 5: sesame oil. The animals were killed 24 h later by decapitation. Blood was collected in order to evaluate plasma levels of LH and FSH. The three weeks post-castration interval was selected for these experiments because previous data of this laboratory had indicated that at this time serum levels of LH and FSH are maximally elevated [19].

Table 1. Effect of 100 μg/rat of progesterone (P), 5α-pregnane-3,20-dione (dihydroprogesterone, DHP), 5α-pregnan-3α-ol-20-one (3α-ol), 5α-pregnan-3β-ol-20-one (3β-ol) on serum levels of LH and FSH of castrated female rats pretreated for 5 days with 0·4 μg ethinyl estradiol (EE) and sacrificed 8 h after progestogen administration

| Treatment | ng LH/ml (NIH-LH-S17) | ng FSH/ml (NIAMD-RAT FSH-RP1) |
|---|---|---|
| 1. EE (0·4 μg) (18)[a] | 3·43 ± 0·39[b,c] | 283·87 ± 7·95[j] |
| 2. EE + P (100 μg) (20) | 8·49 ± 0·82[d] | 1209·46 ± 31·75[k] |
| 3. EE + DHP (100 μg) (18) | 5·87 ± 1·06[e,h] | 566·74 ± 35·01[l] |
| 4. EE + 3α-ol (100 μg) (16) | 8·45 ± 1·16[f] | 603·11 ± 19·84[m] |
| 5. EE + 3β-ol (100 μg) (11) | 16·33 ± 1·71[g,i] | 515·93 ± 12·04[n] |
| 6. OIL (24) | 27·36 ± 1·73 | 1027·24 ± 40·00 |

[a] Number of animals in brackets.
[b] Values are means ± S.E.
[c] 1 *vs* 6: $P < 0·0005$. [d] 2 *vs* 1: $P < 0·0005$. [e] 3 *vs* 1: $P < 0·0125$. [f] 4 *vs* 1: $P < 0·0005$. [g] 5 *vs* 1: $P < 0·0005$. [h] 3 *vs* 2: $P < 0·025$. [i] 5 *vs* 2: $P < 0·0005$. [j] 1 *vs* 6: $P < 0·0005$. [k] 2 *vs* 1: $P < 0·0005$. [l] 3 *vs* 1: $P < 0·0005$. [m] 4 *vs* 1: $P < 0·0005$. [n] 5 *vs* 1: $P < 0·0005$.

*Determination of LH and FSH*

Blood was allowed to clot at room temperature. Sera were separated and kept frozen until assayed for LH and FSH. Serum LH concentrations were determined by the double-antibody radioimmunoassay procedure described by Niswender *et al.*[20]. An antiserum against ovine LH and pure ovine LH iodinated with $I^{131}$ were used. Values have been expressed in terms of NIH-LH-S17. FSH was measured using the NIAMD-RAT-FSH-radioimmunoassay Kit, according to the method of Daane and Parlow[21]. Values have been expressed in terms of NIAMD-RAT-FSH-RP1. Statistical analysis of the data has been performed using Student's "t" test.

## RESULTS

The results in Table 1 summarize the data obtained in the experiments aimed at evaluating the possibility that DHP, 3α-ol and 3β-ol might facilitate the release of pituitary gonadotrophins. It appears that castrated oil-treated female rats have rather elevated levels of serum LH. In agreement with previous data of this and other laboratories these are significantly depressed by the injection of EE [15, 18]. The injection of P on the fifth day of oestrogen priming is followed by a significant increase of serum levels of LH. In animals given P serum levels of LH are more than twice as high as those found in animals not treated with P. DHP, 3α-ol and 3β-ol are all able to significantly elevate serum levels of LH over those observed in animals given EE alone. In this test, 3α-ol appears to be at least as effective as P. DHP, on the contrary, is less effective than P. The activity of 3β-ol is higher than that of any other progestational agent tested.

Table 1 summarizes also the data obtained in the same groups of animals by measuring plasma FSH. First of all it is apparent that the elevated levels of FSH found after ovariectomy can be depressed by the administration of five consecutive daily injections of small doses of EE. This is confirmatory of previous data of this and other laboratories [15, 18]. It is also clear that under the experimental conditions selected P is able to exert a strong positive feedback effect on the release of FSH. The FSH values in EE + P-treated animals are even higher than those found in control animals submitted to castration and treated with oil. DHP, 3α-ol and 3β-ol all exert a stimulating effect on FSH release; such an effect is less pronounced than that of P, and is quantitatively similar for the three steroids.

Table 2 summarizes the data obtained in the experiments in which the negative feedback effect of the different progestational agents on gonadotrophin secretion has been evaluated. It is evident that P, under the conditions of the present experiment, does not inhibit LH release. On the contrary, DHP, 3α-ol and

Table 2. Effect of 2 mg/rat of progesterone (P), 5α-pregnane-3,20-dione (dihydroprogesterone, DHP), 5α-pregnan-3α-ol-20-one (3α-ol), 5α-pregnan-3β-ol-20-one (3β-ol) on serum levels of LH and FSH of female rats castrated since 3 weeks and sacrificed 24 h after progestogen administration

| Treatment | ng LH/ml (NIH-LH-S17) | ng FSH/ml (NIAMD-RAT FSH-RP1) |
|---|---|---|
| 1. P (2 mg) (26)[a] | 21·06 ± 0·97[b] | 939·99 ± 26·82 |
| 2. DHP (2 mg) (21) | 15·21 ± 0·79[c] | 647·03 ± 17·78[g] |
| 3. 3α-ol (2 mg) (25) | 11·48 ± 0·97[d,f] | 545·28 ± 12·75[h] |
| 4. 3β-ol (2 mg) (20) | 16·94 ± 0·91[e] | 1017·47 ± 27·27 |
| 5. OIL (23) | 21·95 ± 0·83 | 916·89 ± 27·15 |

[a] Number of animals in brackets.
[b] Values are means ± S.E.
[c] 2 *vs* 5: $P < 0·0005$. [d] 3 *vs* 5: $P < 0·0005$. [e] 4 *vs* 5: $P < 0·0005$. [f] 3 *vs* 2: $P < 0·0005$. [g] 2 *vs* 5: $P < 0·0005$. [h] 3 *vs* 5: $P < 0·0005$.

3β-ol are all able to significantly depress serum levels of LH. 3α-Ol seems to be more active than either DHP or 3β-ol.

P and 3β-ol do not diminish serum levels of FSH in castrated female rats. On the contrary, DHP and 3α-ol exert a rather strong inhibiting effect on FSH release, 3α-ol being more active than DHP in this respect.

## SUMMARY AND CONCLUSIONS

(1) DHP, 3α-ol and 3β-ol exert a positive feedback effect on LH and FSH secretion qualitatively similar to that of P in castrated oestrogen-primed rats. This observation is compatible with the hypothesis that P exerts its facilitatory effects on gonadotrophin secretion after the local (hypothalamic and pituitary) conversion into DHP and 3α-ol [2–4]. The fact that the activity of 3α-ol is quantitatively similar to that of P in releasing LH suggests that this metabolite might be the intracellular mediator for the positive feedback P exerts on LH release.

(2) The observation that the efficiency of the different P metabolites in releasing LH and FSH is different stresses once more that the central mechanisms which control LH and FSH secretion are not identical [22].

(3) The LH- and FSH-releasing activity of 3β-ol is very interesting. This steroid is not a regular metabolite of P in the P-sensitive structures [3, 11]. However, it is formed in rather large amounts in the ovary, and its concentration in that tissue shows a peak in the afternoon of proestrus at the time of LH and FSH surge [12]. It is consequently possible that this compound plays a physiological role in the processes leading to gonadotrophin release reaching the hypothalamic–pituitary complex *via* the systemic circulation.

(4) P is ineffective in suppressing LH and FSH secretion in castrated rats when given without EE. On the contrary, all 5α-reduced metabolites tested exert some inhibitory effect on gonadotrophin secretion. There are several possible interpretations of these results. One possibility is that P operates on the negative feedback system only after conversion into DHP, 3α-ol, etc. If one accepts such an interpretation, the inactivity of P here reported might be explained by the fact that the short interval used in the experiments did not allow a sufficient conversion of the steroid into its 5α-reduced metabolites. However, such a possibility appears to be a rather remote one. It has indeed been demonstrated that after castration the 5α-reductase activity of the anterior pituitary and of the hypothalamus increases several-fold [23]. Another possibility is that the negative feedback effect of P (in the experimental conditions in which it appears) [24] is a property of the steroid as such. For the reasons just mentioned, the authors would favour such a view, even if, in their experiments, they have been unable to demonstrate a negative feedback effect of P on gonadotrophin release.

(5) The observation that, depending on the experimental conditions, DHP, 3α-ol and 3β-ol may either facilitate or inhibit LH and FSH release is intriguing, and underlines once more the complexity of the feedback mechanisms which control the secretion of gonadotrophins under physiological conditions.

*Acknowledgements*—This work was supported by Grants of the Population Council and of the Ford Foundation, New York, N.Y. The assistance of Drs. A. R. Midgley, Jr., L. E. Reichert, A. F. Parlow and G. D. Niswender, and of the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program, in providing LH and FSH radioimmunoassay materials is gratefully acknowledged. Thanks are also due to Mr. M. Vigliani for his skilful technical assistance.

## REFERENCES

1. Armstrong D. T. and King E. R.: *Endocrinology* **89** (1971) 191–197.
2. Massa R., Stupnicka E. and Martini L.: Excerpta Med. (Amst.) Intern. Congr. Ser. **256** (1972), p. 118.
3. Karavolas H. J. and Herf S. M.: *Endocrinology* **89** (1971) 940–942.
4. Robinsons J. A. and Karavolas H. J.: *Endocrinology* **93** (1973) 430–435.
5. Gloyna R. E. and Wilson J. D.: *J. clin. Endocr. Metab.* **29** (1969) 970–977.
6. Massa R., Stupnicka E., Kniewald Z. and Martini L.: *J. steroid Biochem.* **3** (1972) 385–399.
7. Zanisi M., Motta M. and Martini L.: In *The Endocrine Function of the Human Testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, New York, Vol. 1 (1973) pp. 431–438.
8. Zanisi M., Motta M. and Martini L.: *J. Endocr.* **56** (1973) 315–316.
9. Motta M., Piva F. and Martini L.: *Bull. Swiss Acad. Med. Sci.* **25** (1970) 408–418.
10. Neill J. D. and Smith M. S.: In *Current Topics in Experimental Endocrinology* (Edited by V. H. T. James and L. Martini). Academic Press, New York, Vol. 2 (1974) pp. 73–103.
11. Tabei T. and Heinrichs W. L.: *Neuroendocrinology* **15** (1974) 281–289.
12. Holzbauer M.: *Br. J. Pharmac.* **43** (1971) 560–569.
13. Taleisnik S., Caligaris L. and Astrada J. J.: *J. Endocr.* **44** (1969) 313–321.
14. Taleisnik S., Caligaris L. and Astrada J. J.: *J. Reprod. Fert.* **22** (1970) 89–97.
15. Swerdloff R. S., Jacobs H. S. and Odell W. D.: *Endocrinology* **90** (1972) 1529–1535.
16. Kalra P. S., Kalra S. P., Krulich L., Fawcett C. P. and McCann S. M.: *Endocrinology* **90** (1972) 1168–1176.
17. Brown-Grant K.: *J. Endocr.* **62** (1974) 319–332.
18. Zanisi M. and Martini L.: *Acta endocr., Copenh.* (1975) in press.
19. Zanisi M. and Martini L.: *Acta endocr., Copenh.* (1975) in press.
20. Niswender G. D., Midgley A. R., Jr., Monroe S. E. and Reichert L. E., Jr.: *Proc. Soc. exp. Biol. Med.* **128** (1968) 807–811.
21. Daane T. A. and Parlow A. F.: *Endocrinology* **88** (1971) 653–663.
22. Martini L.: In *Neurosecretion—The Final Neuroendocrine Pathway* (Edited by F. Knowels and L. Vollrath). Springer-Verlag, Berlin (1974) pp. 135–147.
23. Stupnicka E., Massa R. and Martini L.: *Endocrinology* (1975) in press.
24. Davidson J. M.: In *Frontiers in Neuroendocrinology* (Edited by W. F. Ganong and L. Martini). Oxford University Press, New York (1969) pp. 343–388.