Influence of 16α-Hydroxyprogesterone, 20α-Hydroxyprogesterone and 20β-Hydroxyprogesterone on Progesterone Activity in a Bio-Assay (34970)

THOMAS R. FORBES

Department of Anatomy, Yale University School of Medicine, New Haven, Connecticut 06510

Synergisms and antagonisms between progesterone and other naturally occurring steroid hormones have been demonstrated by the application of the Hooker-Forbes bioassay for progestin activity (1–3). The assay is based on a particular biologic reaction, that of the stromal nuclei in the uterus of the ovariectomized mouse (4, 5). The experiments to be reported were undertaken to determine the minimal effective dose (MED) of each of several steroid hormones when individually assayed and then to explore and quantify possible synergisms and antagonisms between progesterone and the other steroid hormones.

Materials and methods. Separate stock solutions of progesterone, 16α-hydroxyprogesterone, 20α-hydroxyprogesterone, and 20β-hydroxyprogesterone were prepared by dissolving weighed amounts of each substance in ether, adding the quantity of sesame oil necessary to supply the desired concentration of the steroid, and removing the ether by distillation.

The MED of progesterone and of 16α-hydroxyprogesterone in the CHI mouse are known (4, 6). Bioassays of these compounds and of 20α-hydroxyprogesterone and 20β-hydroxyprogesterone were carried out in young adult, ovariectomized B6D2F1 mice according to our usual procedure (4). All animals received food and water at will. The MED of a compound was determined by injecting each of a series of progressive dilutions into at least 5 uterine horns. The greatest dilution that would evoke a positive response of the stromal nuclei according to the criteria of the assay (elongated oval outline, conspicuous nucleolus, and fine, evenly scattered chromatin particles) in a majority of the horns in which it was placed was considered to represent the MED for that compound in that strain of mouse.

Mixtures of two compounds were made by combining appropriate dilutions so as to give the desired proportions by weight of the steroids. In the bioassay of such mixtures both the absolute and the relative amount of each hormonal component are important. A series of relative amounts, or proportions, was therefore selected, and a progression of absolute amounts at each proportion was assayed. As in the assay of a single compound, the end point for mixtures of progesterone and another steroid was regarded as the greatest dilution that would evoke a positive response in a majority of at least 5 uterine horns injected with that dilution. In the early stages of an assay the absolute amount of progesterone injected into each horn was several times the MED of that hormone. Thus the ability, if any, of the second steroid to antagonize progestin activity was fully challenged. The assay of one mixture of two steroids in CHI mice is outlined in Table I as an example of the general assay procedure. In the CHI mouse the MED of progesterone alone is 0.2 ng (4), while that of 16α-hydroxyprogesterone alone is 6.0 ng (6). In this assay of a mixture of the two substances, from 10 to 20× the MED of progesterone was always injected. At least 14× the MED was required to overcome the antagonistic effect of the 16α-hydroxyprogesterone present. Thus

[1] This research was supported by Grant 5-R01-HD-00456, National Institute of Child Health and Human Development, USPHS. Trivial names used in this article are progesterone = pregn-4-ene-3,20-dione, 16α-hydroxyprogesterone = 16α-hydroxypregn-4-en-3,20-dione, 20α-hydroxyprogesterone = 20α-hydroxypregn-4-en-3-one, and 20β-hydroxyprogesterone = 20β-hydroxypregn-4-en-3-one.

TABLE I. Dilutions of 2 ng of Progesterone (P): 0.002 ng of 16α-Hydroxyprogesterone (16α), a 1000:1 Mixture, and Response in Uterine Horns Injected (CHI mice).

Dilution	P (ng)	16α (ng)	Assay results
1:1000	2.0	0.0020	+ + − − −
1:900	2.2	0.0022	− − − − −
1:800	2.5	0.0025	− − − − −
1:750	2.7	0.0027	− − − − −
1:700	2.8	0.0028	+ + + + −
1:500	4.0	0.0040	+ + + − −

the progesterone appeared to be exerting only about 7% of its theoretical activity.

If during the course of an assay it became apparent that a steroid was acting synergistically with the progesterone, increasing dilutions of the mixture were injected until the degree of synergism was determined.

In all cases, calculation of the total theoretical number of MED's present in a mixture included those supplied not only by progesterone but also the MED's, if any, supplied by the second steroid. Finally, the theoretical total of MED's divided by the actual total indicated the degree of synergism or antagonism.

A total of 175 uterine horns was injected to establish the MED of the various individual compounds. The assay of 37 mixtures required the injection of an additional 700 uterine horns.

Results. In the B6D2F1 mouse the MED of progesterone was 0.0005 μg; of 16α-hydroxyprogesterone, 2.0 μg; of 20α-hydroxyprogesterone, 0.0004 μg; of 20β-hydroxyprogesterone, 0.00006 μg.

Assays in CHI mice showed no activity in progesterone:16α-hydroxyprogesterone mixtures in proportions of 500:1, 2000:1, 10,000:1, and 50,000:1. A 1000:1 mixture when assayed had 0.07 of its theoretical activity (Table I); a 100,000:1 mixture, 0.1; a 500,000:1 mixture, 0.01.

Progesterone: 20α-hydroxyprogesterone mixtures in proportions of 0.5:1, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 100:1, 1000:1, 10,000:1, 100,000:1, and 500,000:1 in B6D2F1 mice showed no activity on assay, indicating complete antagonism at these proportions.

Assay results in B6D2F1 mice for mixtures of progesterone with 20β-hydroxyprogesterone are shown in Fig. 1. Adjacent points on the graph are connected with straight lines only to assist in visualization; it is not intended to imply that values intermediate between two points necessarily would fall on the line.

Discussion. If the MED of various steroids bioassayed by the Hooker-Forbes method are combined from earlier (4, 6–8) and present results (Table II), it is clear that the CHI and B6D2F1 strains of mice differ somewhat in their sensitivity to a given compound and that the activity of the various hormones relative to each other is not always the same when the two strains are compared. In a third strain of mice, the MED of progesterone was 0.00005 μg (5); in two additional

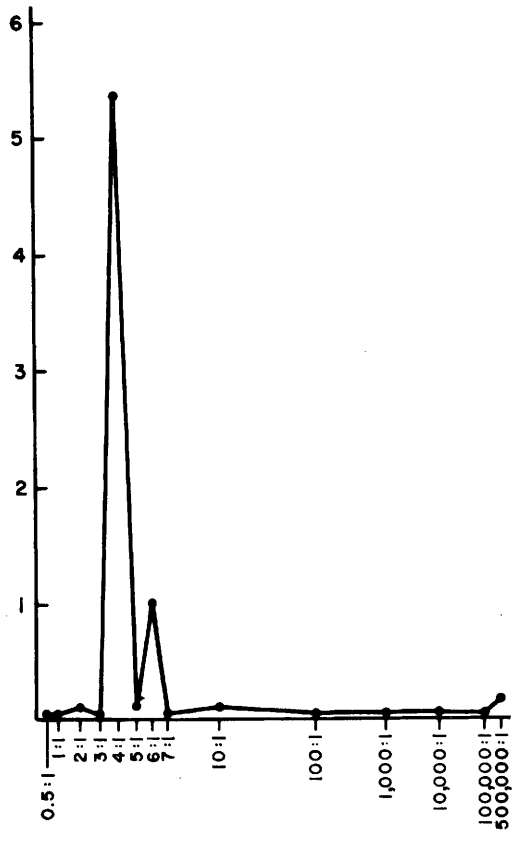

FIG. 1. Assay results for progesterone:20β-hydroxyprogesterone mixtures: The proportions of the mixtures are on the abscissa. On the ordinate, below 1 indicates antagonism; above 1, synergism.

TABLE II. MED (μg) of Various Steroids in Hooker-Forbes Bioassay in Two Strains of Mice.

Mouse strain	Progesterone	16α-OH-prog.	20α-OH-prog.	20β-OH-prog.
CHI	0.0002	0.006	0.0005	0.00010
B6D2F1	0.0005	2.0	0.0004	0.00006

strains the MED was similar to that for the CHI mouse (9, 10).

A limited earlier series of bioassays of progesterone:16α-hydroxyprogesterone mixtures at proportions of 99:1, 9:1, 1:1, and 1:9 had no activity in CHI mice (1). The present assays of this combination give nearly similar results. The earlier assays also showed little activity of progesterone:20α-hydroxyprogesterone and progesterone:20β-hydroxyprogesterone in proportions of 1:1 and 1:9, findings that approximate present findings.

In present and earlier (1–3) studies progesterone:16α-hydroxyprogesterone and progesterone:20α-hydroxyprogesterone showed complete or almost complete antagonism at all proportions that were assayed. Progesterone:estriol mixtures were slightly to completely antagonistic. Mixtures of progesterone with estrone, with estradiol-17β, with testosterone, with 17α-hydroxyprogesterone, and with 20β-hydroxyprogesterone, and progesterone:estrone:estradiol-17β mixtures, although antagonistic at some proportions were also clearly synergistic at other proportions. When synergistic peaks occurred, they were at progesterone:steroid proportions of 1:9 to 99:1 except in the case of estrogens. Progesterone:estrogen mixtures showed peaks of activity at proportions of 50:1 to 33,333:1.

The ability of natural and synthetic estrogens, sometimes in minute proportions, to block a progesterone effect has been noted in other types of bioassay (11–14).

It is tempting to speculate about comparable interactions among endogenous steroids in the intact organism (1–3, 15–17) Antagonism and synergism, whatever their nature, may help to regulate the action of steroid hormones in untreated animals. But it remains to be demonstrated that the transport and perhaps the action of hormones injected for bioassay are the same as for endogenous steroid hormones.

Summary. In the B6D2F1 mouse the MED by Hooker-Forbes bioassay of progesterone was 0.0005 μg; of 20α-hydroxyprogesterone, 0.0004 μg; of 20β-hydroxyprogesterone, 0.00006 μg; of 16α-hydroxyprogesterone, 2.0 μg. Progestin activity of mixtures of progesterone with 16α-hydroxyprogesterone, 20α-hydroxyprogesterone, and 20β-hydroxyprogesterone was measured by the same assay in CHI and B6D2F1 mice. Synergisms, partial antagonisms, and complete antagonisms were exhibited and were shown to depend on the components of the mixtures and their amounts and proportions.

1. Forbes, T. R., Endocrinology **75**, 799 (1964).
2. Forbes, T. R., Endocrinology **79**, 420 (1966).
3. Forbes, T. R., Endocrinology **83**, 411 (1968).
4. Hooker, C. W., and Forbes, T. R., Endocrinology **41**, 158 (1947).
5. Ober, K. G., Klein, I., and Weber, M., Arch. Gynaekol. **184**, 543 (1954).
6. Forbes, T. R., Proc. Soc. Exp. Biol. Med. **125**, 438 (1967).
7. Zander, J. *et al.*, Klin. Wochenschr. **35**, 143 (1957).
8. Zander, J. *et al.*, J. Clin. Endocrinol. Metab. **18**, 337 (1958).
9. Zarrow, M. X., and Neher, G. M., J. Clin. Endocrinol. **13**, 203 (1953).
10. Fujii, K. *et al.*, Bull. Tokyo Med. Dent. Univ. **3**, 225 (1956).
11. Velardo, J. T., and Hisaw, F. L., Endocrinology **49**, 530 (1951).
12. Yochim, J. M., and De Feo, V. J., Endocrinology **71**, 134 (1962).
13. Czyba, J. C., and Dubois, P., C. R. Soc. Biol. **159**, 1572 (1965).
14. Edgren, R. A. *et al.*, Acta Endocrinol. Copenhagen Suppl. **115**, 3 (1967).
15. Dorfman, R. I., Physiol. Rev. **34**, 138 (1954).
16. Forbes, T. R., *in* "Brook Lodge Symposium, Progesterone" (A. C. Barnes, ed.), p. 71. Brook Lodge Press, Augusta, Mich. (1961).
17. Mikhail, G., Zander, J., and Allen, W. M., J. Clin. Endocrinol. Metab. **23**, 1267 (1963).

Received May 8, 1970. P.S.E.B.M., 1970, Vol. 134.