INVESTIGATION OF BLOCK TO GONADOTROPHIN RELEASE IN MATURE FEMALE RATS

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Summary. The block to gonadotrophin release in mature female rats caused by an oestrogen, a progestin and an androgen has been investigated by replacement with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG). Inhibition of ovulation produced by the progestin could be reversed by HCG alone but the effects upon ovulation of oestrogen and androgen were not reversed unless both PMSG and HCG were supplied. The relevance of the results to previous work in the rat and other species is discussed.

INTRODUCTION

Previous workers have attempted to ascertain the mechanism of the inhibition of ovulation produced by gonadal steroids in the female rat (Greep & Jones, 1950; Van Rees, 1961, among others). The reports in the literature, however,

TEXT-FIG. 1. Structural formulae of (a) megestrol acetate, (b) ethinyl oestradiol, and (c) testosterone propionate.

conflict as to whether the action of follicle stimulating hormone (FSH) or of luteinizing hormone (LH) is primarily involved.

Makepeace, Weinstein & Friedman (1937) have shown that inhibition of ovulation in the rabbit by doses of progestin may be reversed with exogenous LH, suggesting that the progestin inhibits the release or production of LH at either pituitary or hypothalamic levels. A similar approach has now been used in the rat in the hope that further information on the mechanism of inhibition of ovulation by a progestin (megestrol acetate), an oestrogen (ethinyl oestradiol) and an androgen (testosterone propionate) might result (Text-fig. 1).

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MATERIALS AND METHODS

Virgin, specific pathogen-free, albino rats (200 to 250 g), obtained from our own colony, were given aureomycin (50 mg/l) in the drinking water as a prophylactic for 1 week before, and for the duration of, the experiment (Bennett, Vallance & Vickery, 1967).

Each steroid under examination was administered daily to groups of 5 to 10 animals for 14 days. Megestrol acetate and ethinyl oestradiol were given orally as suspensions in an aqueous medium composed of 1.2% w/v carboxy methyl cellulose, 1.5% w/v Tween 80 and distilled water to 100%. The dose volumes were adjusted to 25 ml/kg body weight. Testosterone propionate was administered subcutaneously twice daily (09.00 hours and 16.30 hours) as a solution in a 1:4 mixture of ethyl oleate and arachis oil. The dose volume was adjusted to 2 ml/kg body weight. Each animal was also injected intraperitoneally with a 1% aqueous solution of Dianil blue 2 R (3 ml/rat) on Days 5 to 8 of treatment inclusive (Bennett *et al.*, 1967) while pregnant mare serum gonadotrophin, PMSG ('Pabyrn', Paines & Byrne) and/or HCG ('Pregnyl', Organon) were administered intraperitoneally on Days 9 and 11 of the experiment, respectively, to some of the steroid-treated groups. All animals were killed on Day 15 of the experiment and the ovaries inspected for the occurrence of ovulation indicated by the presence of colourless or pink corpora lutea on the otherwise dark blue ovaries.

RESULTS

It can be seen from Table 1 that complete inhibition of ovulation was obtained with megestrol acetate at a dose of 12 mg/kg/day and almost complete inhibition

TABLE 1
EFFECT OF HCG ON OVULATION INHIBITION PRODUCED BY
MEGESTROL ACETATE AND BY ETHINYL OESTRADIOL IN THE RAT

Compound	Dose (mg/kg/day)*	HCG dose (i.u./rat)	% rats ovulating	Mean no. ovulations/ rat ovulating
Megestrol acetate	12	—	0	0
Megestrol acetate	12	10	100	9.0
Ethinyl oestradiol	2	—	20	4.0
Ethinyl oestradiol	2	10	20	2.5

* Ten rats were used/dose group.

of ovulation with ethinyl oestradiol at 2 mg/kg/day. When, however, HCG (10 i.u.) was administered to animals already receiving megestrol acetate, all the animals ovulated, the mean number of ovulations per rat being in agreement with figures obtained for spontaneous ovulation in our colony. The rats which received HCG as well as ethinyl oestradiol, however, showed no such return to normal. The number of animals ovulating was not increased and the mean number of ovulations per rat ovulating showed no significant difference from the mean number of ovulations resulting from dosing with ethinyl oestradiol alone.

The results of a further examination of ethinyl oestradiol are shown in Table 2. In this experiment 2 mg/kg/day completely inhibited ovulation, and it is

TABLE 2
EFFECTS OF PMSG AND HCG ON OVULATION INHIBITION
PRODUCED BY ETHINYL OESTRADIOL (2 MG/KG/DAY) IN THE RAT

<i>PMSG dose (i.u./rat)</i>	<i>HCG dose (i.u./rat)</i>	<i>No. of rats/ group</i>	<i>% rats ovulating</i>	<i>Mean no. of ovulations/ rat ovulating</i>
—	—	14	0	0
2.5	—	8	0	0
5.0	—	8	0	0
10.0	—	5	0	0
20.0	—	9	0	0
40.0	—	10	20	2.5
2.5	10.0	9	90	6.7
5.0	10.0	7	85	6.8
10.0	10.0	9	90	10.9
20.0	10.0	6	100	26.5
40.0	10.0	8	100	53.5
—	10.0	9	0	0

evident that injections of both PMSG and HCG were necessary to overcome this inhibition. A dose of PMSG between 5 and 10 i.u. given in conjunction with 10 i.u. of HCG was required to produce normal ovulation. Doses of PMSG alone, up to 40 i.u., produced only a limited number of ovulations in a small proportion of the animals and no ovulations were seen after administration of HCG alone. High doses of PMSG in conjunction with HCG gave rise to superovulation.

Table 3 shows that both PMSG and HCG were necessary to reverse the inhibition of ovulation caused by testosterone propionate (0.2 mg/kg twice daily).

TABLE 3
EFFECT OF ADMINISTRATION OF PMSG AND HCG ON THE
INHIBITION OF OVULATION PRODUCED BY TESTOSTERONE
PROPIONATE (0.2 MG/KG TWICE DAILY) IN THE RAT

<i>PMSG dose (i.u./rat)</i>	<i>HCG dose (i.u./rat)</i>	<i>No. of rats/ group</i>	<i>% rats ovulating</i>	<i>Mean no. of ovulations/ rat ovulating</i>
—	—	10	0	0
5.0	—	10	10	1.0
5.0	10.0	8	100	10.4
—	10.0	10	0	0

DISCUSSION

Human chorionic gonadotrophin specifically possesses luteinizing activity (Parlow, 1961). It does not show any follicle-stimulating activity by the augmentation test of Steelman & Pohley (1953). On the other hand, PMSG possesses predominantly FSH activity, but with a small amount of contaminant

LH activity. Thus Catchpole (1964) was able to show in hypophysectomized females that both interstitial cells and follicles are stimulated by PMSG. The presence of contaminant LH activity has been demonstrated in the present experiments by the fact that a large dose of PMSG alone (40 i.u.) produced ovulation and luteinization in some animals treated with ovulation-inhibiting doses of ethinyl oestradiol although follicular development evidently occurred at much lower doses.

The results of the present study suggest, therefore, that megestrol acetate produces its anti-ovulatory effect purely by inhibition of LH, thus differing from ethinyl oestradiol and testosterone propionate which exert their effects by inhibition of both LH and FSH. The results of Wolfe (1946) and Greep & Jones (1950) suggest a similar conclusion in the case of progesterone, although Selye, Browne & Collip (1936) and Van Rees (1959) concluded that both FSH and LH are affected by the natural hormone. Supporting evidence for the similarity in mechanism of inhibition of ovulation between megestrol acetate and progesterone is given by Kobayashi, Kobayashi, Takezawa, Oshima & Kawamura (1962).

That ethinyl oestradiol and testosterone propionate appear to inhibit ovulation by a similar mechanism is perhaps not surprising since a survey of the literature reveals no well substantiated difference between the effects of oestrogens and androgens on the pituitary-ovarian axis of the female. Thus, although oestrogens have been reported not to produce any change in pituitary FSH levels (Paesi, de Jongh, Hoogstra & Engbelbregt, 1955, Hoogstra & Paesi, 1957) this does not agree with the work of Greep & Jones (1950) who found that oestrogens gave rise to atretic follicles in the ovary suggesting decreased FSH release from the pituitary. One explanation for these contradictory results is that oestrogens may inhibit both release and synthesis of FSH, and that the resultant pituitary content is the same as in the normal animal. Testosterone propionate has also been reported to produce atrophy of follicles by Greep & Jones (1950), although here increased FSH content of the pituitary is implicated (Greep & Jones, 1950; Hoogstra & Paesi, 1957).

Oestrogens and androgens are reported to decrease the LH content of the pituitary in females (Laqueur & Fluhmann, 1942; Hellbaum & Greep, 1946; Chowder & McCann, 1965). Greep & Jones (1950), however, conclude that androgens increase pituitary LH, although closer analysis of their results reveals a slight decrease in LH content of the pituitary at the higher doses of the androgen.

In the male, on the other hand, several workers agree that oestrogens have a significantly different effect from androgens: oestrogens decrease and androgens increase the FSH content of the pituitary (Paesi *et al.*, 1955; Hoogstra & Paesi, 1957; Van Rees, 1961). There does not seem to be any published information on comparative effects of oestrogens and androgens on levels of LH in the male.

From the results of the present experiment it is suggested that testosterone propionate exerts an effect on the gonad-pituitary axis in the female different from that in the male rat. The effect in the former is identical with that produced by oestrogen administration so that, although there are three structural gonadal hormones, they may be characterized in the female into the two functional

groups possessing oestrogenic and progestagenic activities. A possible explanation of this phenomenon could be that androgens are converted to oestrogens in the ovary, suggested by results obtained by Bresloff, Oakey & Stitch (1966).

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