MECHANISM OF ACTION OF MEDROXYPROGESTERONE (17 α -ACETOXY-6 α - METHYL PROGESTERONE) IN THE RAT

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Summary. In an attempt to characterize the effects of medroxyprogesterone (17 α -acetoxy-6 α -methyl progesterone) more precisely, the compound was administered subcutaneously to immature rats twice a week (12.5 mg/injection) for 5 weeks. The effects of medroxyprogesterone on the pituitary gland were evaluated by measuring the LH content of the gland by the method of Parlow, while those on the ovaries were studied by measuring the ovarian response to exogenous PMS (10 i.u.). In the treated animals there were significant decreases in ovarian, uterine, adrenal, pituitary and body weights; corpora lutea were absent and vaginal opening was delayed. The ovarian response to PMS was not decreased. There was a significant decline in both concentration and total gland content of LH due to treatment.

It is suggested that the major action of medroxyprogesterone, when administered for an extended period of time in the doses used, is exerted at the pituitary level. It blocks the synthesis of LH in the pituitary gland without affecting ovarian sensitivity to gonadotrophins.

INTRODUCTION

There is a degree of uncertainty about the precise mechanism by which synthetic progestagens achieve control of fertility. Although it is widely believed that they primarily act at the pituitary–hypothalamic level and suppress the discharge of gonadotrophins (Pincus, 1965), other evidence indicates that peripheral action on the ovary and fallopian tubes may play a significant role. The evidence in support of the latter view consists of near normal excretion of pregnanediol in a certain percentage of medicated cycles (Goldzieher, Moses & Ellis, 1962), a lack of decrease in the excretion of gonadotrophins as judged by mouse uterine weight assay (Loraine, Bell, Harkness, Mears & Jackson, 1963, 1965), and the occurrence of ovulation in rats treated with progestagens for an extended period of time (Pincus & Merrill, 1961; Holmes & Mandl, 1962; Wakeling, 1965). When a specific assay for luteinizing hormone (LH) such as the ovarian ascorbic acid depletion (OAAAD) test of Parlow (1961) is used a significant diminution in the peak of urinary LH associated with ovulation has

been noted (Stevens, Vorys, Besch & Barry, 1965). This still leaves unanswered the question whether progestagens block the release of LH or its synthesis in the pituitary gland. Effects of progestagen on the pituitary gland may involve a decrease in output of LH from the gland without affecting its synthesis; or a decline in the output may be secondary to a decrease in LH synthesis. Thus, reduced output of LH may not necessarily represent inhibition of release. Bio-assay of LH in the pituitary gland by a method which discriminates between LH and FSH may provide at least a partial answer to this problem.

It was, therefore, decided to study the effects of medroxyprogesterone (17α -acetoxy-6 α -methyl progesterone, 'Provera') on the pituitary gland by measuring the LH content of the gland. A possible action of the compound at ovarian level was also studied. Since this compound has very little inherent oestrogenic action (Duncan, Lyster & Clark, 1963), observed effects can largely be attributed to its progestational properties.

MATERIALS AND METHODS

Immature female Holtzman rats weighing between 50 and 55 g on Day 21 of life were divided into two groups. One group was injected subcutaneously with an aqueous suspension of medroxyprogesterone (Depo-Provera, Upjohn) twice a week (12.5 mg/injection) for 5 weeks, while the other served as a control. At frequent intervals during the course of the study the animals were weighed and examined for vaginal opening.

The animals were killed at the end of 5 weeks and anterior pituitary glands, adrenals, uteri and ovaries were weighed. All weights were expressed on a 100-g body-weight basis. The number of corpora lutea in the ovaries was also recorded. The pituitaries were kept frozen until the time of assay.

Three days before autopsy five rats from each group were injected with a total dose of 10 i.u. of pregnant mares' serum gonadotrophin (PMS; Equinex, Ayerst). The hormone was injected subcutaneously once a day for 3 days. The rats were killed the day following the last injection. The ovarian and uterine weights were recorded. Their pituitaries were not assayed.

LH assay

The OAAD test of Parlow (1961) was used. Holtzman female rats, weighing between 55 and 60 g on Day 24 of life, were injected with 50 i.u. of PMS on Day 26, followed by 50 i.u. of human chorionic gonadotrophin (HCG; A.P.L., Ayerst) 60 to 65 hr later. A week following HCG injection 2 mg of wet pituitary tissue dissolved in 1 ml of saline was injected through a tail vein. Three hours $\pm$ 10 min following the injection the ascorbic acid concentration in both ovaries was determined by a modification of the method of Mindlin & Butler (1938).

One to three glands, selected randomly from each group, were pooled. Two rats/pool were used. The assays were run in two replicates. Each replicate contained one to two pools of control pituitaries, two pools of treated pituitaries, and two doses (0.8 and 3.2 μ g) of reference standard (NIH-LH-ovine-s-3). In the preliminary trials it was found that dose-response curves for rat pituitary LH and standard LH were parallel.

RESULTS

Ovaries

The ovaries of the treated animals were significantly smaller than those of the controls (Table 1). They contained no grossly visible corpora lutea. In contrast, the ovaries from control animals contained normal-looking corpora.

TABLE 1
EFFECTS OF MEDROXYPROGESTERONE TREATMENT ON VARIOUS ORGAN WEIGHTS
(mg/100 g BODY WEIGHT)

	No. of animals	Body weight	Ovarian weight	Uterine weight	Adrenal weight	Pituitary weight
Control	11	180 ± 4†	35.5 ± 1.4	204 ± 16	30.8 ± 0.8	5.03 ± 0.40
Medroxy-progesterone treated	7	160 ± 3***	11.0‡ ± 0.3***	123 ± 34§**	9.2 ± 0.1***	3.06** ± 0.45

** $P < 0.01$; *** $P < 0.001$.

† Mean ± standard error.

‡ Ovaries lacked corpora lutea.

§ Includes one rat with deciduoma.

The ovarian response to exogenous PMS also appeared different in the two groups (Table 2); the percentage increase in the ovarian weight caused by PMS injection in the progestagen-treated animals was almost twice as much as that in the control animals. Thus, no evidence for a decline in the ovarian sensitivity to gonadotrophins was found.

TABLE 2
OVARIAN RESPONSE (mg/100 g BODY WEIGHT)
TO EXOGENOUS PMS (10 i.u.)

	Control		Medroxyprogesterone	
	None	PMS	None	PMS
No. of animals	11	5	7	5
Mean ovarian weight (mg)	35.5	50.7	11.0	21.6
Absolute increase (mg)		15.2		10.6
Percentage increase		42.4		96.4

Body weight

The body weights of the treated animals showed a significant decline (Text-fig. 1). The rate of gain/day in the control animals was 4.24 g as compared to 3.02 g in the treated animals. Thus, the growth rate of the treated animals was about 25% lower than that of the controls.

Vaginal opening

In the control group 38% of animals had closed vaginae when they were 35 days old. In contrast, 100% of the treated animals showed closed vaginae on

this day. By Day 53 of life, almost all the treated animals had opened vaginae (Text-fig. 1).

Uterine weight

Out of a total of twelve animals treated with medroxyprogesterone, two showed spontaneous deciduomata at autopsy. One of the animals had been treated with PMS. Whether this animal is included in the mean or not, the mean uterine weight of the treated animals was significantly lower than that of the controls. A similar difference was present following PMS treatment.

TEXT-FIG. 1. A comparison between control (---) and medroxyprogesterone treated (—) animals with respect to body weight (●) and percentage of closed vaginae (○).

LH level

Both the concentration and the total content of LH in the pituitary gland were reduced significantly by medroxyprogesterone treatment (Table 3). The treatment also caused a significant decrease in the pituitary weight (Table 1).

The adrenal glands of the treated animals showed pronounced atrophy (Table 1).

DISCUSSION

Theoretically, there are three possible explanations of the observed effects of medroxyprogesterone on the ovary. The lack of corpora lutea could be due to: (i) inhibition of LH release from the pituitary gland; (ii) a peripheral action of the compound, inhibiting the effect of gonadotrophins on the ovary; or (iii) a combination of these two possibilities.

The ovarian response of the treated animals to PMS observed in this study does not support the action of medroxyprogesterone at the ovarian level. In fact the

data suggest that there may have been some increase in the ovarian sensitivity to PMS. Eckstein & Mandl (1962) obtained similar evidence with norethynodrel and suggested that it might have been due to the inherent oestrogenic activity of this compound. However, since medroxyprogesterone is very weak in this respect (Duncan *et al.*, 1963), such an explanation will not suffice. Other evidence in hypophysectomized rats (Duncan *et al.*, 1963) and in women (Johannisson, Tillinger & Diczfalusy, 1965) is also against medroxyprogesterone's having a peripheral action on the ovary.

The lack of ovulation, delay in vaginal opening and significant decline in uterine weight in the treated animals seem to be due to inhibition of LH discharge from the pituitary gland. Since this decrease in LH output was not accompanied by a build-up of LH in the gland, but by a significant decline both

TABLE 3
EFFECT OF MEDROXYPROGESTERONE ON THE CONCENTRATION
AND CONTENT OF LH[†] IN THE RAT PITUITARY

	Control	Medroxyprogesterone
No. of pituitary pools [‡] assayed	3	4
Pituitary weight (mg/100 g body weight)	5.03	3.06**
LH concentration (μ g/mg wet pituitary)	1.93 $\pm$ 0.39	0.39 $\pm$ 0.12**
LH content (μ g/gland/100 g body weight)	9.70	1.19

** $P < 0.01$.

[†] Ovarian ascorbic acid depletion test of Parlow (1961).

[‡] One to three glands/pool.

in LH concentration and content, medroxyprogesterone seems to have blocked synthesis of this hormone. A decline in the LH discharge may be secondary to a decline in LH synthesis. This may be due either to direct effects of medroxyprogesterone on the pituitary gland or an inhibition of the hypothalamic neurohumoral factor involved in LH synthesis or release. Obviously, assay of this factor might help to define more precisely the action of progestagen on the pituitary gland.

This action of medroxyprogesterone appears to differ from that of its parent compound—progesterone. The latter causes a significant build-up of LH in the pituitary gland, suggesting that it causes a decrease in LH release but not in its formation in the gland (Hoffman & Schwartz, 1965; van Rees & de Groot, 1965). On the other hand, 17 α -acetoxy progesterone, which differs from medroxyprogesterone in not having a 6 α -methyl group, also causes a significant decline in pituitary LH level when injected into pregnant rats in doses exceeding 1 mg/day (Morrisette, McDonald & Morrison, 1964).

The pronounced adrenal atrophy in the treated animals confirms the observation of others in the rat (Glenn, Richardson & Bowman, 1959; Holub, Katz & Jailer, 1961). The latter authors found adrenal atrophy primarily due to

inhibition of ACTH synthesis in the pituitary gland. Thus, the action of medroxyprogesterone on the pituitary gland appears to involve inhibition of the synthesis of at least two trophic hormones—ACTH and LH. A significant decline in body weight in the treated animals could be due to a similar action of medroxyprogesterone on the synthesis of growth hormone. However, the possibility of a decrease in food intake due to the treatment should not be overlooked (Pincus, 1965).

The ovarian atrophy found in the medroxyprogesterone-treated rats suggests an inhibition of follicle-stimulating hormone (FSH) release in addition to that of LH. However, whether it also blocks the synthesis of FSH is unknown since the pituitary level of this hormone was not studied. Morrisette *et al.* (1964) obtained suggestive evidence for an increased level of pituitary FSH in pregnant rats treated with 17α -acetoxypregesterone in doses exceeding 1 mg/day.

A significantly lower mean uterine weight in the medroxyprogesterone-treated group indicates that the oestrogen output from the ovaries was reduced as a sequel to inhibition of LH discharge from the pituitary gland. The presence of spontaneous deciduomata in two treated animals and an increase in uterine weight following PMS treatment suggest that uterine sensitivity was not affected by medroxyprogesterone. In ovariectomized rats medroxyprogesterone can support the development of deciduomata (Pincus & Merrill, 1961).

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