Evidence for Progestogen Secretion by ACTH-Stimulated Adrenals.* (20644)

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One method for testing the luteotropic potency of lactogenic or mammotropic hormone (MH) has been to stimulate the luteal tissue of hypophysectomized rats to form progestogen, which in turn induces a deciduoma to form in areas of endometrial trauma(1). Such a test has also been used in detecting luteotropic activity in rat placenta(2,3). However, in light of the very extensive studies on the many different steroids including progestogens that may be formed by the adrenal cortex(4,5) it has become necessary to ascertain whether or not adrenocorticotropic hormone (ACTH), by way of progestogenic adrenal steroids, might be responsible for deciduoma formation.

In earlier studies(1) the precaution of removing the adrenals had been taken in proving a luteotropic function for MH. It is now possible to report that ACTH preparations highly potent as adrenocortical growth stimulants are responsible for progestogen formation in the absence of the ovaries.

Procedure. The test animals were immature female Long-Evans rats hypophysectomized at approximately 30 days of age and injected for one week thereafter.† For luteotropic assays, it has been necessary to inject the rats prior to hypophysectomy with an ovulatory or luteinizing dose of pituitary or chorionic gonadotropins(1). A single injection of pregnant mare serum gonadotropin (PMSG) 4 days before hypophysectomy has served this purpose. Luteal tissue so induced shows functional cell hypertrophy after hypophysectomy only when stimulated with MH or rat placental extracts. Even when large masses of luteal tissue were induced in these immature ovaries they were incapable of secreting progestogen in the absence of the hypophysis or the hormones with luteotropic potency. The fractions to be tested for luteotropic activity were injected for 7 days beginning on the day of hypophysectomy. On the fourth day of injection a thread was inserted in one uterine horn; and necropsy was performed on the day following the last injection. In testing for ACTH-induced progestogen, neither pre-treatment with PMSG nor the presence of the ovaries was found to be necessary. The futility of attempting to gain even the slightest hint of the quality or quantity of progestogens formed by ACTH-stimulated adrenals under these circumstances was fully appreciated. Nevertheless, 2 available progestogenic steroids, progesterone and desoxycorticosterone acetate (DOCA) were tested for deciduoma-inducing potency. The pituitary, ovaries and adrenals may all be dispensed with when testing these substances.

Results and discussion. Table I shows the results obtained in a series of rats, injected with highly purified preparations of beef growth hormone (somatotropin), and sheep ACTH and MH. None of the controls injected with the ACTH vehicle (5% beeswax in peanut oil), and none of the somatotropin-treated animals showed deciduoma formation. This was true in both groups even when heavily luteinized ovaries had developed in response to PMSG prior to hypophysectomy. The somatotropin was estimated to have contained about 1% ACTH on the basis of adrenal histology. The MH contained less than an estimated 1% of ACTH; and activated the induced luteal tissue in daily subcutaneous doses of 100 γ (aqueous), or 50 γ in beeswax. As in an earlier series(1) the adrenals could be removed on the day of thread-insertion without interfering with MH-induced deciduoma formation. The ACTH preparations

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† This test animal may be employed in assaying the 6 well known anterior lobe hormones, using histologic criteria in the thyroid, adrenal, ovary and tibia for their detection.

TABLE I. Deciduoma Formation and Adrenal Weights in Immature Hypophysectomized Rats Injected with Various Hormones.

Hormone	Daily dose, mg	No. of rats	Positive deciduoma	Avg wt l. adrenal, mg
None		27	0	4.3
MH-1	1.0	10	10	4.6
	.2	3	3	4.0
	.1	5	5	—
*	.05	3	2	4.4
ACTH-1	.060	3	3	19.4
"	.045	12	5	20.2
"	.03	3	0	15.5
2	.06	3	3	35.0
"	.06	3	0	—†
"	.03	7	3	21.2
3	.01	3	0	6.3
"	.03	3	3	22.5
4	.03	3	0	15.3
5	.03	3	0	12.5
6	.20	4	0	11.7
Somatotropin	.45	7	0	7.5
	.10	5	0	6.2
	.02	4	0	4.8
Progesterone	2.0	5	5	—
	1.0	3	3	—
DOCA	2.5	4	4	—
	1.0	3	1	—
Hydrocortisone	5.0	3	0	—

Notes: (1) All of the somatotropin and MH animals had been pretreated with PMSG. (2) All of the ACTH and one MH preparation (marked *) were inj. in 5% beeswax in peanut oil. (3) Avg wt of left adrenal in 12 normal rats, 32-36 days old, was 12 mg. † Adrenalectomized.

represent different fractions prepared by methods to be described elsewhere(6). Briefly ACTH-2 and -3 were obtained at a more advanced step in the purification procedure than ACTH-1. They were slightly more potent in adrenal weight increasing activity than ACTH-1; and had been subjected to peptic digestion, a procedure that destroys MH. ACTH-6 had been treated in such a way as to preserve a good ascorbic-acid depleting potency equal to that of ACTH-1, and to render it a relatively weak adrenal weight increasing preparation (Table I).

It was observed that ACTH usually induced deciduoma formation in those rats in which the weight of the adrenal had increased several fold over that of the average hypophysectomized control figure of 4.3 mg. However, some animals with the weight of the left adrenal as low at 15.8 mg showed deciduomata; whereas some with a weight as high as 23 mg did not.

In the greatly enlarged adrenals the sinusoids showed marked congestion with disruption of the reticuloendothelial lining and extravasation of blood in small scattered areas. The normally regular parenchymal pattern was greatly distorted by the rapid growth, vascular engorgement and necrosis. Some of the degenerated cells contained nuclear remnants, sudanophilic lipoids and birefringent material that was yellowish, not pink, in Sudan-stained sections. It appeared as though the adrenocortical parenchyma had become in part a holendocrine gland liberating whole cells and products of degenerated cells into the blood stream. Some normal-appearing cortical cells were seen also in the sinusoids of the medulla.

The abnormal appearance of some of the over-stimulated adrenals suggested that ACTH may have promoted the liberation into the blood stream of an unusual assortment and quantity of cholesterol derivatives. However, the probability remains that this might also be possible in a moderately stimulated, but non-pathological adrenal.

Deciduoma formation in the ACTH-treated rats was always associated with practically complete atrophy of the thymus and an average body weight loss of one g per day. The thymus could only be detected as a minute lymphoid remnant by staining the surrounding fat tissue in toto in alum carmine and clearing with methyl salicylate. The thymolytic effect is interpreted as being due to corticoids such as cortisone. Progesterone and DOCA were not found to be potent thymolytic agents; nor did they cause body weight loss such as has been observed with cortisone and hydrocortisone.

As shown in Table I both DOCA and progesterone induced deciduoma formation in the hypophysectomized immature rat when given alone and without pre-treatment with estrogen, in daily doses of one mg. This could probably be considered a borderline progestogenic dose of DOCA; but Astwood (7) has shown that as little as 0.1 mg (optimum 0.75 mg) of progesterone per day may induce deciduomata in pseudo-pregnant adult rats oophorectomized, but not hypophysecto-

mized. Cortisone and hydrocortisone have never been considered progestogenic, and in this experiment the latter proved inactive in deciduoma tests when given as the acetate at the daily dose of 5 mg.

Pending identification and quantitation of the steroids formed in the ovary under the influence of MH, and in the adrenal following injections of ACTH by chromatographic technics(4,5,8,9) it may be a reasonable assumption that in equivalents of DOCA the daily effective secretion would be in the neighborhood of one mg—and of progesterone, somewhat less. However, it would seem more important to emphasize the fact that many steroids with different androgenic, estrogenic, progestogenic, etc., potencies may be formed in ACTH-stimulated adrenals (4,5,10,11), and that therefore the formation of a deciduoma would depend not only upon the total amount of progestogens secreted, but also upon the amounts of other steroids formed, *e.g.*, estrogens which, in optimal doses, enhance this action of progestogen and in high doses inhibit it(7,12,13).

Conclusions. 1. Daily doses of 30 γ of an ACTH suspension in beeswax and peanut oil injected for a week stimulated the adrenals of immature hypophysectomized and oophorectomized rats to form progestogen(s) as shown by the deciduoma test. 2. MH in daily doses as low as 50 γ accomplished this effect in immature hypophysectomized-adrenalectomized rats, the ovaries of which had previously been luteinized with PMSG. 3. Progesterone and DOCA in 7 daily doses of one mg induced deciduoma formation in immature hypophysectomized rats; hydrocortisone in 7 daily doses of 5 mg did not. 4. Since both MH and ACTH induce progestogen secretion, an assayist should study the respective target tissue of each (corpus luteum or adrenal cortex) for histologic evidence of activation rather than use biologic or chemical evidence of progestogen formation. An alternative would be to test for MH progestogen in hypophysectomized-adrenalectomized animals, and for ACTH-progestogen after hypophysectomy and oophorectomy.

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