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DEPRESSION OF DECIDUAL GROWTH IN THE RAT WITH LH ANTISERUM AND PITUITARY AUTOTRANSPLANTATION

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Summary. Rabbit antiserum to sheep LH inhibited uterine growth in adult rats following uterine trauma on Day 4 of pseudopregnancy. The weights of the horns subjected to trauma were similar to those obtained in rats ovariectomized at the time of trauma and given only progesterone, and in rats with the adenohypophysis transplanted beneath the kidney capsule on the day of ovulation. Antiserum to LH given to rats with an autotransplanted pituitary did not alter decidual growth. Oestrone restored maximal uterine growth in rats receiving LH antiserum and in rats with an autotransplanted pituitary. Simultaneous injections of FSH and LH resulted in maximal decidual cell response in pituitary autotransplanted rats, while either, given alone, had only slight stimulatory effects. It is suggested that LH antiserum inhibits the oestrogen production necessary for maximal uterine growth. Luteinizing hormone does not appear to be necessary, unless in minute quantity, for production of progesterone in amounts large enough to support maximal uterine growth after trauma.

INTRODUCTION

Mechanical or chemical trauma to the endometrium on the 4th day after vaginal cornification in the pseudopregnant rat results in massive decidua formation (for review see Shelesnyak, 1957). The importance of oestrogens and progesterone in the regulation of maximal sensitivity of the rat uterus for optimal deciduoma formation has been demonstrated by DeFeo (1963) and Yochim & DeFeo (1962). Considerable data are available to illustrate the action of specific pituitary hormones on ovarian steroid production in the rat; however, little information is available concerning the indirect effect of pituitary hormones on the decidual cell response (DCR). The present studies with LH antiserum and pituitary autografts were initiated to investigate pituitary hormone regulation of ovarian steroidogenesis and the subsequent effect of ovarian steroids on the DCR.

MATERIAL AND METHODS

Animals

Adult, virgin female rats of the Holtzman strain, weighing 220 to 280 g, were used. Animal quarters were kept at 22 to 27° C with 14 hr of fluorescent illumination and 10 hr darkness per day. The midpoint of the light period was 15.00 hours. Purina Lab Chow and tap water were given. Daily vaginal lavage was carried out to determine the stage of the oestrous cycle. At least two complete oestrous cycles of 4 or 5 days were completed before initiation of any treatment.

Hormones

The luteinizing (LH) and follicle stimulating (FSH) hormones used were dissolved in sterile water and 0·25 to 0·5 ml were injected at intervals of 12 ± 2 hr. Crystalline oestrone and progesterone were dissolved in sesame oil and 0·5 ml were injected daily (24 ± 3 hr intervals). Rabbit antiserum to sheep LH was produced by giving three bi-weekly injections of 1·5 mg of the antigen (LH) dissolved in 1·5 ml saline mixed with an equal volume of Freund's complete adjuvant (Difco, Detroit, Michigan). This was followed by two bi-weekly booster injections of 2·0 mg of antigen alone. Antiserum samples were collected 1 to 2 weeks after the last injection and pooled. Control serum was collected from rabbits receiving only adjuvant. All sera were frozen in small quantities until the time of injection, when a portion was thawed and used and the remainder refrigerated for a period not exceeding 5 days. Antiserum to LH was tested for its ability to block ovulation in pro-oestrus-hypophysectomized rats given exogenous LH. A test for purity was run on Ouchterlony gel diffusion plates (Ouchterlony, 1953) and antibody titre was measured by interfacial test (Kabat & Mayer, 1961). Antiserum injections were given subcutaneously at intervals of 12 ± 2 hr.

Procedures

Pseudopregnancy was induced on the morning of vaginal cornification (Day 0) by giving the cervix two 15-sec stimulations with a metal rod attached to an electric toothbrush. Day 1 of pseudopregnancy was characterized by the first marked appearance of leucocytes in the vaginal lavage (di-oestrus) following cervical stimulation.

Uterine trauma consisted of midventral laparotomy at midday of Day 4 (±1 hr) and insertion of a sharp needle into the lumen of the right uterine horn from cervix to oviduct, scratching the antimesometrial endometrium. The left horn served as a control. Rats showing vaginal cornification before Day 4 were not subjected to trauma and those having nodular or incomplete decidua formation in the injured horn on Day 9 were discarded.

Hypophysectomy was performed on the day of vaginal cornification by parapharyngeal approach under ether anaesthesia. The adenohypophysis was autotransplanted beneath the left renal capsule immediately after removal. Before hypophysectomy, all rats had the left oviduct removed so that ovulation could be verified by observing ova in the excised oviduct. Any rat that had not

ovulated was not hypophysectomized. The hypophysial fossa was examined for evidence of hypophysial fragments and when fragments were suspected, the skulls were prepared for histological examination.

Collection of data

All rats were killed on Day 9, the uteri were removed, and split at the bifurcation, trimmed and each horn weighed to the nearest 0·1 mg on a Mettler Gram-o-Matic balance. Horns which had been subjected to trauma were then dried for 24 hr in a convection oven and weighed. Ovaries, when present, were collected, weighed and fixed for histological examination.

TABLE 1

EFFECT OF LH ANTISERUM OR ANTISERUM WITH OESTRONE OR PROGESTERONE ON UTERINE WEIGHT (DAY 9) IN INTACT PSEUDOPREGNANT RATS AFTER UTERINE TRAUMA (DAY 4)

Treatment group	*No. rats*	*Weight of horn subjected to trauma*			*Wet weight of control horns*[a]	*Ovaries*[a]
		Wet[a]	*Dry*[a]	*% Dry*		
Control serum[b] (Days 1 to 5 or 4 to 8)	10	1838·9 ± 67·7	293·9 ± 10·0[c]	15·1[c]	159·0 ± 11·8	55·9 ± 2·2
LH antiserum (Days 1 to 5)	5	853·0** ± 120·6	—	—	129·1 ± 11·7	48·0 ± 4·1
LH antiserum (Days 4 to 8)	12	1024·2** ± 49·9	170·5** ± 9·8[d]	16·7[d]	120·3** ± 5·3	46·1** ± 1·1
LH antiserum + 3 mg progesterone (Days 4 to 8)	5	1584·5* ± 65·0	252·1* ± 11·5	15·9	126·5* ± 7·6	46·6* ± 2·4
LH antiserum + 1 μg oestrone (Days 4 to 8)	5	2041·8 ± 106·2	304·4 ± 12·8	15·0	170·3 ± 10·4	48·9 ± 4·9

[a] Numbers represent the average values (mg) ± S.E. for each group.
[b] Serum injections (0·1 ml) for all groups were given twice daily and steroid injections once daily.
[c] Based on the average weight of five rats.
[d] Based on the average weight of seven rats.
* ($P<0·05$), ** ($P<0·01$) Significantly different from the controls.

RESULTS

Antiserum tests

The antibody titre of the LH antiserum was approximately 1:20,000, indicating that 1 ml of pooled antisera should neutralize 625 mg of LH. A gel diffusion test of the antiserum revealed a heavy precipitin band with sheep LH and a light band to FSH. Eight mg of LH consistently induced ovulation in hypophysectomized pro-oestrous rats; ovulation was blocked if 40 μl of LH antiserum was given at the time of LH injection but was not blocked with 10 μl. Ovulation in intact pro-oestrous rats was blocked with 10 μl of LH antiserum, but not with 5 μl. All injections were given intravenously.

Effects of LH antiserum

Rabbit antiserum to sheep LH significantly lowered ($P<0·01$) both the wet and dry weights of injured horns on Day 9 when injected on either Days 1 to 5

or 4 to 8 of pseudopregnancy in intact rats. The weight of the control horns was also significantly lowered when antiserum was given on Days 4 to 8 (Table 1). Uterine weights after treatment with LH antiserum resembled those in ovariectomized rats given only progesterone (Table 2) and in rats with an autotransplanted pituitary (Table 3). Antiserum did not alter uterine growth in ovariectomized rats which had been given progesterone and oestrone (Table 2) or in rats with an autotransplanted pituitary (Table 3). Antiserum had very little influence on DCR when given on Days 1 to 4 if the rats were then ovariectomized and given progesterone and oestrone; however, antiserum given during the pre-trauma period did prolong the 'sensitive period' of the uterus to trauma as indicated by the results of trauma on Day 5 and subsequent ovariectomy and steroid replacement (Table 2).

TABLE 2

EFFECT OF OESTRONE, PROGESTERONE AND LH ANTISERUM (ALONE OR IN COMBINATION) ON UTERINE GROWTH AFTER UTERINE TRAUMA IN OVARIECTOMIZED PSEUDOPREGNANT RATS

Treatment group[a]	*Day of ovariectomy*[b]	*Weight of horn subjected to trauma*[c]		
		Wet	*Dry*	*% Dry*
Progesterone + oestrone (Days 4 to 8)	4	1774·4 ± 61·0	269·8 ± 8·3	15·2
LH antiserum + oestrone and progesterone (Days 4 to 8)	4	1846·3 ± 152·2	281·4 + 22·2	15·3
LH antiserum (Day 1 to 4) progesterone and oestrone (Days 4 to 8)	4	1658·7 ± 131·4	251·0 ± 19·4	15·1
Oestrone (Days 4 to 8)	4	339·8** ± 43·4	59·40** ± 5·9	17·9
Progesterone (Days 4 to 8)	4	1072·9** ± 86·4	171·5** ± 13·2	16·0
Progesterone and oestrone (Days 5 to 8)	5	283·9** ± 31·1	49·1** ± 4·0	17·3
LH antiserum (Days 1 to 5) progesterone and oestrone (Days 5 to 8)	5	1200·8** ± 168·3	168·8** ± 22·8	14·1

[a] Oestrone (1 μg) and progesterone (3 mg) were given once daily whether alone or together; antiserum (0·1 ml) was given twice daily.
[b] Ovariectomy and uterine trauma were performed at the same time on the day indicated.
[c] Values given are average weights (mg) ± S.E. for five rats killed on Day 9.
** ($P < 0·01$) Significantly different from progesterone + oestrone (Days 4 to 8).

Histologically, the ovaries of rats receiving antiserum had fewer large vesicular follicles than those receiving control serum, although when antiserum treatment was stopped on Day 5 some follicular recovery was noted on Day 9.

Steroid replacement

Daily injections of oestrone (1 μg) and progesterone (3 mg) were capable of supporting uterine growth associated with maximal DCR when given to pseudopregnant rats after ovariectomy and uterine trauma on Day 4 (Table 2). Progesterone (1073 mg) supported uterine growth but not to the same extent as

progesterone with oestrone (1774·4 mg). Oestrone alone (339·8 mg) did not support a positive DCR. The average horn weight (1093·3 mg) in rats with an autotransplanted pituitary was similar to that (1072·9 mg) obtained in ovariectomized rats given only progesterone; but oestrone given daily after trauma increased uterine growth to the level noted in intact pseudopregnant rats (2064·9 compared with 1838·9 mg). Daily injections of oestrone also counteracted the depressing effect of LH antiserum on uterine growth after trauma in intact pseudopregnant rats (Table 1). Progesterone significantly increased ($P<0·01$) uterine weight in intact rats receiving LH antiserum but not to the extent of oestrone (1584·5 compared with 2041·8 mg).

Gonadotrophin replacement after pituitary autotransplantation

Injections of FSH or LH had a significant ($P<0·05$) stimulatory effect on DCR in rats with autotransplanted pituitaries; if the two gonadotrophins were given together, maximal DCR was observed (Table 3). The weights of the intact

TABLE 3

EFFECT OF LH ANTISERUM, GONADOTROPHINS AND OESTRONE ON UTERINE WEIGHT (DAY 9) AFTER UTERINE TRAUMA (DAY 4) IN RATS WITH AN AUTOTRANSPLANTED PITUITARY[a]

Treatment group[b]	*No. rats*	*Weight of horn subjected to trauma*[c]			*Wet weight of control horns*	*Ovaries*
		Wet	*Dry*	*% Dry*		
Control	5	978·0 ± 119·1	151·9 ± 18·8	15·5	112·5 ± 5·3	49·3 ± 2·7
LH antiserum (Days 4 to 8)	5	1093·3 ± 96·6	167·4 ± 13·4	15·4	116·6 ± 7·5	48·2 ± 2·0
Oestrone (Days 4 to 8)	6	2064·9** ± 170·1	316·8** ± 25·0	15·4	170·7** ± 8·4	47·2 ± 2·2
FSH (Days 0 to 8)	5	1338·7* ± 55·9	209·4* ± 13·1	15·6	129·2 ± 7·7	53·3 ± 1·8
LH (Days 0 to 8)	5	1360·7* ± 108·8	204·6 ± 16·4	15·0	129·0 ± 11·7	49·5 ± 2·3
FSH and LH (Days 0 to 8)	5	2033·8** ± 174·8	295·7** ± 22·3	14·6	216·1** ± 21·2	55·4 ± 1·3

[a] Autotransplantation was performed on Day 0 and uterine trauma on Day 4. All rats were killed on Day 9.

[b] Oestrone (1 μg) was given once daily; LH antiserum (0·1 ml), FSH (20 μg) and LH (5 μg) were given twice daily.

[c] Numbers represent the average values (mg) ± S.E. for each group.

* ($P<0·05$), **($P<0·01$) Significantly different from the controls.

uterine horns were significantly increased ($P<0·01$) by oestrone or FSH and LH in combination, but not by FSH or LH alone. The size of the ovarian follicles in those rats is reflected in the ovarian weights shown in Table 3; but no significant differences are present. In all rats, corpora lutea which appeared to be functional were observed. Only small ovarian vesicular follicles remained at Day 9 in rats with autotransplanted pituitaries unless they received FSH, or FSH and LH.

In all groups, decidual growth represented an increase in both the dry and wet weight of the horns. Antiserum increased the percentage dry matter of the

horns subjected to trauma and decreased the wet weight of the control horns in intact rats. A decrease in wet weight of the control horns was generally associated with a lack of oestrogen.

DISCUSSION

The present data suggest that LH antiserum interferes with oestrogen production in the pseudopregnant rat, thereby decreasing uterine growth after massive trauma. Support for this interpretation lies in the fact that LH antiserum given to intact pseudopregnant rats after trauma reduced uterine growth to the weight observed in ovariectomized rats given only progesterone and rats with autotransplanted pituitaries. The autotransplanted pituitary apparently produces an uninterrupted secretion of prolactin (Everett, 1956) but almost completely loses its ability to secrete FSH and LH (Nikitovitch-Winer & Everett, 1958). Under these circumstances, a significant amount of ovarian follicular oestrogen secretion would not be expected, but progesterone is secreted (Everett, 1956). Oestrone was capable of producing maximal uterine growth in intact rats given LH antiserum, ovariectomized rats given progesterone with LH antiserum and rats with an autotransplanted pituitary. Antiserum given during the pre-trauma period was capable of extending the duration of the 'uterine sensitive period', a phenomenon attributed to decreased oestrogen level (Yochim & DeFeo, 1963). Furthermore, LH antiserum did not decrease uterine growth in rats with an autotransplanted pituitary, suggesting that progesterone secretion was not greatly affected by LH antiserum treatment.

Antiserum to LH, prepared in a similar manner to the present antiserum, neutralized endogenous LH in the male rabbit (Quadri, Harbers & Spies, 1966), inhibited ovarian ascorbic acid depletion by LH in the rat (Hoppe, Quadri & Spies, 1967) and suppressed the secretion of endogenous oestrogen in the rabbit (Spies & Quadri, 1967).

The ability of the uterus to form decidua in response to trauma apparently depends on both absolute and relative levels of oestrogen and progesterone (Yochim & De Feo, 1963). Present data concerning pituitary autotransplantation or administration of LH antiserum during the pre-trauma period with administration of oestrone after trauma demonstrates that maximal uterine growth can be obtained with relatively low pre-trauma levels of oestrogen if adequate progesterone and oestrogen are present during the post-trauma period. An 'oestrogen surge', as postulated for decidua formation in intact pseudopregnant rats (Shelesnyak, 1960) or mice (Finn, 1966) after an intra-uterine injection of oil, may not be necessary for maximal decidua formation after massive uterine trauma. Yochim & DeFeo (1963) obtained optimal DCR following massive trauma in ovariectomized rats given constant levels of oestrogen and progesterone, and a suboptimal DCR can be obtained even in the absence of oestrogen (Finn, 1965; Greenwald, 1958; Meyer & Cochrane, 1962).

In the present experiment, LH antiserum depressed uterine growth more when given after trauma than before trauma. This was true except when LH antiserum was given on Days 1 to 5 in intact rats, indicating that antibodies can exist for several days in circulation (Quadri *et al.*, 1966; Schwartz & Gold,

1967). Madhwa Raj, Sairam & Moudgal (1968) prevented implantation in the rat with a single injection of LH antiserum on the morning of the 4th day of pregnancy. They suggested that LH antiserum prevented the oestrogen surge by preventing an earlier LH surge which, in turn, was responsible for oestrogen secretion. An injection of oestrogen on the evening of the same day reversed the effect of the LH antiserum and allowed implantation to take place. These data are compatible with the oestrogen surge theory but do not rule out other possibilities that the oestrogen level remains relatively constant but is too low for implantation to occur. The oestrogen requirement for blastocyst- or oil-induced decidua formation appears to be different from that resulting from massive trauma (Finn, 1965). Hetherington (1968) demonstrated a difference in decidua formation following an intra-uterine injection of air compared with oil. Different hormonal requirements for DCR appear to be related to the type of uterine stimulus and it is therefore difficult to correlate the present results with those using a different stimulus for decidua formation.

The essential rôle played by FSH and LH in DCR appears to be to promote ovarian oestrogen production. Luteinizing hormone stimulates progestin production from luteal tissue in several species including the rat (Major, Armstrong & Greep, 1967). Large amounts of LH are apparently not necessary for the secretion of enough progesterone to support maximal uterine growth after trauma, since rats with autotransplanted pituitaries had maximal uterine growth when given only oestrone after trauma. Since FSH was ineffective in inducing maximal DCR in rats with autotransplanted pituitaries, it appears that FSH prepares the follicle for oestrogen production and LH induces the oestrogen production necessary for maximal DCR. Apparently, prolactin from the pituitary autograft stimulated the secretion of sufficient progesterone for maximal DCR.

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