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Effects of Ovarian Hormones on Foetal and Placental Growth in the Mouse

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Abstract

The effects of ovariectomy and treatment with progesterone and oestradiol on foetal and placental growth were examined. The occurrence of cellular hypertrophy and hyperplasia in the placenta in response to treatments was determined by measuring protein, DNA and RNA : DNA ratios. In control mice, which had not been ovariectomized, the daily administration of 0·01–2·56 μg oestradiol on days 10–15 of pregnancy caused only small decreases in the number of live foetuses and foetal and placental weights on day 16. When mice were ovariectomized on day 10, gestation was maintained by administering 5 mg progesterone daily, but terminated when only 1 mg progesterone was given daily. However, when ovariectomized mice receiving 1 mg progesterone were also given 0·01 μg oestradiol on days 10–15, gestation was maintained and the number of live foetuses and foetal and placental weights on day 16 were normal. Increasing the daily dose of oestradiol up to 2·56 μg in mice receiving 1 mg progesterone on days 10–15 had no effect on foetal and placental growth. In contrast, the daily administration of 0·64–2·56 μg oestradiol to ovariectomized mice receiving 5 mg progesterone on days 10–15 terminated gestation in some mice and considerably reduced the number of live foetuses and foetal and placental weights in those mice which remained pregnant on day 16.

None of the treatments increased protein, DNA or the RNA : DNA ratio in the placenta to levels above those seen in control mice. The results are discussed in relation to ovariectomy-induced placental overgrowth in the rat.

Introduction

Ovariectomy-induced placental overgrowth has been studied extensively in the rat (Butterstein and Leathem 1970, 1972; Callard and Leathem 1971; Csapo and Csapo 1973; Csapo and Wiest 1973). This placental 'hypertrophic' response reaches maximal values when ovariectomy is performed between days 10 and 12 of gestation and pregnancy is maintained by giving progesterone alone or together with low doses of oestradiol (Csapo and Csapo 1973; Csapo and Wiest 1973). The increased size of the placentae results principally from cellular hyperplasia rather than hypertrophy, with little or no change in protein:DNA and RNA:DNA ratios (Butterstein and Leathem 1970). Administration of progesterone and/or oestrogens to intact pregnant rats fails to elicit placental overgrowth (Butterstein and Leathem 1972; Csapo and Csapo 1973; Bartholomeusz and Bruce 1976). Yochim and Zarrow (1961) and Csapo and Csapo (1973) have reported that placental overgrowth in ovariectomized rats is accompanied by small but significant increases in foetal weight, but Callard and Leathem (1971) did not observe any associated increases in foetal growth. Ovariectomy also induces placental overgrowth in the rabbit (Abdul-Karim *et al.* 1971; Bruce and Bartholomeusz 1976). However, it remains unclear whether this pheno-

menon occurs in mammals other than the rat and rabbit, and the physiological significance of ovariectomy-induced placental overgrowth, in relation to the maintenance of gestation and foetal growth, is unknown (Callard and Leathem 1971; Chan and Leathem 1977). In the study reported here the effects of ovariectomy and exogenous progesterone and oestradiol on foetal and placental growth were examined in the mouse during days 10–16 of pregnancy. To facilitate the interpretation of data for placental weight, total protein, DNA and RNA:DNA ratios in the placenta were also determined.

Materials and Methods

Experiment 1

Randomly bred female mice of the Quackenbush strain (University of Sydney Animal House, Castle Hill) were housed four to a box with a single fertile male and with lights on between 0600 and 1800 h. Mice were examined each morning before 0900 h and those with vaginal copulatory plugs were removed and designated day 1 pregnant. These mice were allocated at random to groups to be killed at approximately 1400 h on days 14, 16 and 18 of pregnancy. Parturition in this strain usually occurs between 1800 h on day 19 and 0800 h on day 20. Animals were killed by cervical dislocation, the uteri were promptly dissected and the numbers of live, dead and resorbing foetuses were recorded. A small proportion of mice did not become pregnant after mating and were discarded. Both horns of each uterus were carefully slit lengthwise and live foetuses and the corresponding placentae were dissected and placed in ice-cold 0·9% (w/v) NaCl solution. Care was taken to remove the attached foetal membranes from each placenta. The pooled foetuses and placentae were gently blotted and weighed and mean foetal and placental weights for each mouse were determined. The placentae were stored in 0·9% (w/v) NaCl solution at −18°C for subsequent biochemical analyses.

The pooled placentae from each mouse were homogenized in 6·0 ml ice-cold distilled water, and RNA, DNA and protein were precipitated by the prompt addition of 2·0 ml 1·0 M $HClO_4$ to each homogenate. The sample was centrifuged at 1000 *g* for 20 min and the supernatant was discarded. The $HClO_4$-insoluble pellet was washed once in 10·0 ml 0·2 M $HClO_4$. All procedures were carried out at 2–5°C. RNA and DNA were extracted from the $HClO_4$-insoluble pellet by hydrolysis at 90°C for 20 min in 20·0 ml 0·5 M $HClO_4$, and were recovered in the supernatant obtained by chilling and centrifuging the sample at 1000 *g* for 20 min. The $HClO_4$-insoluble pellet remaining after the extraction of RNA and DNA was solubilized by heating at 90°C for 60 min in 20·0 ml 0·25 M NaOH, and aliquots were taken to determine protein. RNA, DNA and protein were each estimated in duplicate by the methods of Mejbaum (1939), Burton (1956) and Hartree (1972), using yeast RNA, calf thymus DNA and bovine serum albumin respectively as standards. Mean foetal weight and placental weight, protein, DNA and RNA : DNA ratio were determined for each day of pregnancy (Table 1) from the corresponding means for individual mice, so that for each day of pregnancy mice were weighted equally and no attempt was made to correct these data for variation due to litter size. Almost all mice had a total litter size (live, dead and resorbing foetuses) of nine or more. The significance of differences between days was determined by Student's *t*-test (Steel and Torrie 1960).

Experiment 2

Mice were mated and designated day 1 pregnant as in experiment 1. All mice were anaesthetized on day 10 with pentobarbitone sodium given intraperitoneally and the uterine horns were examined via dorso–lateral incisions in the abdominal wall. Mice which were not pregnant were discarded. Pregnant mice were allocated at the time of laparotomy to 18 treatment groups at random, and those in groups 7–18 were promptly ovariectomized. Animals received daily doses of progesterone and/or oestradiol on days 10–15, according to the treatment schedules shown in Table 2, and were killed on day 16. Progesterone and oestradiol were given by separate subcutaneous injections each in 0·1 ml peanut oil. Mice which were not ovariectomized (groups 1–6) received no progesterone. All mice were laparotomized, received hormone injections and were killed at around 1400 h, except that those mice receiving 5·0 mg progesterone daily (groups 13–18) were given two injections each of 2·5 mg progesterone in 0·1 ml peanut oil per day, at about 0700 and 1500 h.

Foetal weight and placental weight, protein, DNA and RNA : DNA ratio were determined as in experiment 1. Mice with a total litter size (live, dead and resorbing foetuses) on day 16 of less than nine were rejected, in order to minimize variation in foetal and placental weight due to litter size during the period of hormone treatment. (Accurate determination of litter size at the time of laparotomy was not possible.) Only eight mice distributed over five treatment groups (or about 5% of those killed on day 16) were rejected on this basis. Results were obtained for eight mice in each treatment group, and mean values for each group were calculated as in experiment 1. The significance of differences between treatment groups was tested by analysis of variance and Student's *t*-test.

Results

In experiment 1 foetal weight increased rapidly between days 14 and 18, but placental weight increased by only 33% between days 14 and 16 and did not change between days 16 and 18 (Table 1). The small changes in placental protein, DNA and RNA:DNA ratio which occurred between days 16 and 18 were not significant. The total number of foetuses per mouse in experiment 1 averaged 10·8, and 95% of all foetuses were alive when the mice were killed. Additional groups of 6, 8 and 5 mice were anaesthetized and sham-ovariectomized on days 9, 10 and 12 respectively of pregnancy. This procedure did not cause any significant changes in numbers of live foetuses or mean foetal and placental weights during late pregnancy.

Table 1. Growth of the foetus and placenta during late pregnancy in the mouse

Results are expressed as means ± s.e.m., and are calculated from the means for individual mice

Day of pregnancy	No. of mice	Number of live foetuses per mouse	Total No. of foetuses per mouse[A]	Weight of live foetus (mg)	Placenta[B]			
					Wet weight (mg)	Protein (mg)	DNA (μg)	RNA:DNA ratio
14	9	9·0±1·2	10·1±1·0	131±4	74·3±3·0	6·15±0·51	315±19	1·78±0·05
16	11	11·2±0·6	11·3±0·6	426±16	99·5±3·8	8·11±0·26	364±10	1·76±0·03
18	6	10·3±1·0	10·8±1·1	1010±29	101·8±3·1	9·10±0·25	368±15	1·61±0·07

[A] Live, dead and resorbing foetuses. [B] Data are from the placentae of live foetuses only.

In experiment 2 (Table 2) the administration of oestradiol to intact mice did not reduce significantly the number of live foetuses per mouse or mean foetal weight on day 16 ($P > 0·05$, groups 2–6 *v.* group 1). However, increasing the dose of oestradiol did cause small decreases in these two parameters ($P < 0·01$, linear effect of dose, groups 2–6). Oestradiol treatment caused only small changes in placental weight, protein, DNA and RNA:DNA ratio in mice in groups 1–6. Of these changes, only the decreases in wet weight and DNA were significant ($P < 0·05$, groups 2–6 *v.* group 1). When mice were ovariectomized on day 10 and given progesterone only, the maintenance of pregnancy was dependent upon progesterone dose (group 7 *v.* group 13). Gestation was terminated in all mice receiving 1 mg progesterone alone daily. In a few of these mice some of the foetuses were observed to be aborted on days 12 and 13, and the size and appearance of resorbing foetuses which remained in the uterus on day 16 indicated that all foetuses died early after the time of ovariectomy. Foetal and placental weights in mice receiving 5 mg progesterone daily were normal (group 13 *v.* group 1).

Table 2. Effects of ovariectomy and treatment with progesterone and oestradiol on foetal and placental growth in the mouse

Results are expressed as means ± s.e.m., and are calculated from the means for individual mice. All mice were laparotomized on day 10 and killed on day 16

Group	Treatment on day 10	Hormone treatment on days 10–15: daily doses		Number of mice with live foetuses[A]	Number of live foetuses per mouse[B]	Weight of live foetus (mg)	Placenta[C]			
		Progesterone (mg)	Oestradiol (μg)				Wet weight (mg)	Protein (mg)	DNA (μg)	RNA : DNA ratio
1	Laparotomy only	—	—	8	13·1±1·0	425±18	103·3±2·3	8·22±0·24	379±11	2·06±0·08
2		—	0·01	8	13·8±0·6	423±22	95·9±3·5	7·93±0·22	339±16	2·11±0·06
3		—	0·04	8	13·3±0·8	449±11	94·8±3·5	7·73±0·25	336±14	2·24±0·07
4		—	0·16	8	10·8±1·5	412±21	89·6±3·5	7·63±0·54	333±13	2·17±0·03
5		—	0·64	8	10·4±0·7	379±16	96·0±2·9	7·59±0·25	371±9	1·87±0·05
6		—	2·56	8	9·6±1·9	364±13	92·0±3·7	7·36±0·34	358±14	1·89±0·06
7	Ovariectomy	1·0	—	0	—	—	—	—	—	—
8		1·0	0·01	6	12·0±1·3	389±19	92·2±4·3	8·53±0·51	355±19	1·98±0·04
9		1·0	0·04	7	10·9±1·6	411±23	93·1±2·8	7·81±0·50	347±13	2·05±0·04
10		1·0	0·16	7	11·9±0·9	383±26	93·9±3·4	8·37±0·42	350±10	2·01±0·05
11		1·0	0·64	8	11·0±1·3	383±20	97·0±5·4	8·39±0·60	381±30	1·93±0·05
12		1·0	2·56	6	11·2±1·3	388±16	93·7±4·1	8·85±0·46	395±16	1·82±0·07
13	Ovariectomy	5·0	—	7	11·0±1·4	421±18	100·9±2·1	8·33±0·07	364±11	2·12±0·06
14		5·0	0·01	7	12·4±0·7	435±17	101·6±3·6	8·39±0·43	369±17	2·11±0·05
15		5·0	0·04	7	11·6±1·7	375±20	95·0±2·3	8·06±0·31	350±14	2·13±0·05
16		5·0	0·16	7	7·3±1·2	309±16	70·9±4·0	6·54±0·51	297±29	1·92±0·07
17		5·0	0·64	4	5·0±2·1	280±22	66·8±1·4	6·32±1·20	274±31	2·05±0·09
18		5·0	2·56	5	2·6±0·5	307±24	75·8±3·7	6·62±0·77	339±38	2·06±0·12

[A] Out of eight mice pregnant on day 10. [B] Means for mice with live foetuses only. [C] Data are from the placentae of live foetuses only.

When mice received 1 mg progesterone plus 0·01 μg oestradiol daily, normal numbers of live foetuses and foetal weights were obtained in six out of eight mice, and placental overgrowth did not occur (group 8 *v.* group 1). In further groups of ovariectomized mice which all received 0·025 μg oestradiol daily, gestation was maintained in a majority of those given 0·5 mg progesterone but in none given only 0·25 mg progesterone daily (unpublished data). Increasing the daily dose of oestradiol from 0·01 to 2·56 μg did not change the number of live foetuses or foetal and placental weights in mice which received 1 mg progesterone daily ($P > 0·05$, linear effect of dose, groups 8–12). In contrast, when 0·16–2·56 μg oestradiol was given to mice receiving 5 mg progesterone daily (groups 16–18) gestation was terminated in several mice and in those remaining pregnant on day 16 the number of live foetuses had decreased markedly ($P < 0·001$). The size and appearance of dead and resorbing foetuses indicated that foetal mortality occurred both early and late during the interval between ovariectomy and day 16. The mean weight of the remaining live foetuses was reduced to around 300 mg ($P < 0·001$). Placental weight, protein and DNA were all reduced ($P < 0·01$) but the RNA:DNA ratio in these placentae remained very similar to that in control mice (groups 16–18 *v.* group 1).

Discussion

Day 10 was chosen for ovariectomy in experiment 2 on the basis of preliminary trials which showed that the administration to intact mice of a wide range of doses of oestradiol from this stage of gestation onwards resulted in little reduction in numbers of live foetuses. Prior to days 10–12 of pregnancy in the rat, foetal survival is very sensitive to the level of oestrogen treatment (Csapo and Csapo 1973), and preliminary trials in the mouse showed that when oestradiol treatments commenced early after implantation on days 5 and 6 the proportion of foetuses that survived to day 12 or later was highly variable. Spontaneous placental overgrowth occurs in rats due to low litter size (Csapo and Csapo 1973), and in strains of mice with litter sizes ranging between 4 and 10 there is a significant negative regression of placental weight on litter size (McLaren 1965). In groups 1–15 in experiment 2 the numbers of live foetuses in pregnant mice were uniformly high, and the effects of variation in litter size on the placentae were presumably small. The changes in placental weight which occurred in groups 16–18 were opposite to those which might have been expected due to the low live litter sizes in these groups.

In the rat placental DNA synthesis and accumulation cease on about day 17, whereas there are large increases in the amounts of RNA and protein between days 17 and 19 (Winick and Noble 1966). The data in Table 1 suggest that such a pattern of cellular hyperplasia followed by cellular hypertrophy does not occur in mouse placentae, since, although there was a small increase in placental protein between days 16 and 18, the RNA:DNA ratio actually decreased slightly between days 14 and 18. Since placental weight did not increase beyond day 16 in experiment 1, mice were killed at this stage of pregnancy in experiment 2. In order to ensure that placental overgrowth did not occur in ovariectomized and hormone-treated mice only after day 16, additional groups each of six ovariectomized mice received different dose combinations of progesterone and oestradiol daily until day 18. In no instance did mean placental weight in the treated groups of mice increase significantly over that in control animals. The mean placental weight in the control group on day 18 was 98 mg, and the highest mean among the treated groups was 101 mg.

The ability of administered progesterone to maintain gestation in ovariectomized mice in the absence of exogenous oestrogen appears to depend upon the amount administered, the time of ovariectomy, and probably differences between strains. When mice are ovariectomized on day 14, gestation is maintained in a majority of those receiving 1 mg progesterone daily, but 0·5 mg per day is inadequate in most mice (Hall 1957). When ovariectomy was performed on day 10 (experiment 2) or on days 6 or 9 (unpublished data) 1 mg progesterone per day was uniformly ineffective. It is not known why 5 mg but not 1 mg progesterone maintained gestation in experiment 2. If the presence of small amounts of oestrogen is essential for the maintenance of pregnancy in these mice, then the extra-ovarian sources of oestrogen in mice receiving 1 mg progesterone daily must have been inadequate. Giving 5 mg progesterone daily may have reduced the requirement for oestrogen or increased adrenal oestrogen secretion. It is possible, also, that with the higher dose there was a significant conversion of the administered progesterone to oestrogen. In further preliminary studies gestation was maintained in some but not all ovariectomized mice by giving 0·004 μg oestradiol plus 1·0 mg progesterone daily. This amount of oestradiol is less than one-millionth of the daily dose of progesterone given to mice in group 13 in experiment 2, and the possibility that the batch of progesterone employed contained oestrogenic contaminants at this level cannot be excluded.

A curious finding in experiment 2 was that whereas the daily administration of 0·16–2·56 μg oestradiol had little effect on the maintenance of gestation and foetal growth in intact mice or in ovariectomized mice receiving 1 mg progesterone daily, it did seriously interfere with foetal survival and growth in ovariectomized mice receiving 5 mg progesterone daily. In these mice (groups 16–18) the placentae of the remaining live foetuses were smaller and contained fewer cells, which, however, were of about normal size judging from the relative amounts of protein, RNA and DNA which these and control placentae contained. Thus the amount of placental hyperplasia which normally occurs between days 10 and 16 was diminished in groups 16–18. Why a combination of 5 mg progesterone and 0·16–2·56 μg oestradiol should have this effect is not known. The mouse placenta can metabolize progesterone to C-19 steroids with androgenic activity (Okker-Reitsma 1976), and it is possible that more placental androgen was formed in mice in groups 16–18. However, androgens have no direct toxic effect on the foetus in the rat (Marois 1966), and foetal survival and growth were normal in additional groups of ovariectomized mice which received 0·1 or 1·0 mg testosterone propionate in addition to 1 mg progesterone and 0·04 μg oestradiol daily (unpublished data).

In view of the striking increases in placental growth that are induced in rats by ovariectomy it was surprising that placental overgrowth was not observed in any of the treated mice in the present study. It remains possible that overgrowth might have been elicited with other ovarian hormone treatments, but this seems unlikely in view of the ranges of doses of oestradiol and progesterone employed. On the basis of studies relating plasma oestrogen concentrations to degree of placental overgrowth, it has been proposed that placental growth is regulated in the rat by the plasma concentrations of both oestrogen and a distinct and as yet unidentified ovarian placental growth inhibitor (Csapo and Csapo 1973; Csapo and Wiest 1973). However, the response of the pregnant rat to ovariectomy and steroid hormone administration varies considerably from one strain to another, as regards pregnancy maintenance and the extent of placental overgrowth (for a discussion see Callard and Leathem

1971). Csapo and Csapo (1973) have suggested that placental overgrowth in the ovariectomized rat may be directly related to improved pregnancy maintenance, but the possible mechanism involved remains unknown (Chan and Leathem 1977). The present results show that normal litter sizes and rates of foetal growth are maintained in ovariectomized mice in the absence of placental overgrowth. It is possible that ovariectomy induces a functional change in these placentae, but the present findings for placental RNA, DNA and protein do not suggest any such change. The data also suggest that the mouse ovary does not secrete a placental growth inhibitor during the latter half of gestation and that, provided minimal amounts of oestrogen are present, the peripheral plasma oestrogen concentration has little influence on placental growth in the mouse.

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