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Do Oestrogens Regulate Placental Growth in the Mouse?

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Abstract

Some effects of litter size, ovariectomy and oestradiol (E_2) on placental growth were determined. In order to increase variation in litter size, some mice had one oviduct removed and the remainder were unilaterally ovariectomized 3–4 weeks prior to mating. Thus after mating mice became unilaterally pregnant. Growth was examined qualitatively by measuring changes in placental DNA, protein, RNA : DNA ratios and *in vivo* incorporation of [5-^{3}H]uridine into RNA. Spontaneous placental hyperplasia due to low litter size occurred to an equal extent in intact mice and mice which were ovariectomized on day 10 of pregnancy and given 1·0 mg progesterone (P) and 10 ng E_2 daily. Ovariectomy on day 10 increased the incorporation of [5-^{3}H]uridine into placental RNA on day 16 in mice with six or more foetuses. When mice were ovariectomized on day 5 and given 1·0 mg P and 10 or 60 ng E_2 daily to maintain gestation, rates of placental growth remained similar to the rate in control, intact mice. The results suggest that, unlike the case in the rat, oestrogens do not regulate placental growth in the mouse.

Introduction

In addition to mechanical and maternal nutritional factors, placental growth in the rat is regulated by the plasma concentration of oestrogens. When rats are ovariectomized at mid-pregnancy and gestation is maintained by giving daily doses of progesterone (P) alone, the weights of the placentae increase by up to 80% above those in control, intact rats. The overgrowth comprises primarily cellular hyperplasia with little change in RNA : DNA and protein : DNA ratios, and involves both the labyrinth and basal portions of the placentae. The amount of placental overgrowth may be regulated by adjusting the daily dose of oestradiol (E_2) given to ovariectomized rats, and if sufficient E_2 is given no overgrowth occurs (see Hayashi 1977 for a review). It has been more difficult to demonstrate E_2 regulation of placental growth in the intact animal. Giving exogenous E_2 to intact rats with 7–12 foetuses does not influence placental growth. However, the spontaneous placental hyperplasia that occurs in intact rats with 1–6 foetuses may be prevented by the administration of E_2 (Csapo and Wiest 1973), and Chan and Natoli (1979) increased placental growth in intact rats by giving injections of antiserum to E_2 at around day 10 of pregnancy. These authors proposed that E_2 acts directly on the placenta to suppress protein, RNA and DNA synthesis. Croskerry and Dobbing (1978) have suggested that the level of ovarian oestrogen secretion after days 10–12 of pregnancy is regulated by the mass of placental tissue present and hence litter size, via the action of placental luteotropin.

The daily administration of a wide range of doses of E_2 to both intact mice and mice ovariectomized on day 10 of pregnancy does not change rates of placental growth in animals with nine or more foetuses (Miller 1978). These findings suggested a basic difference between the mouse and rat, as regards the ovarian regulation of placental growth. However, because of the indirect evidence cited above that litter size regulates plasma E_2 levels and hence placental growth in the intact rat, it remained possible that a role for E_2 could be demonstrated in the mouse by contrasting the regression of placental growth on litter size in groups of animals which remained intact or were ovariectomized on day 10 and given constant daily doses of P and E_2; i.e. if placental growth in the mouse is regulated by E_2 then the regression slopes should differ between intact and ovariectomized animals if, as seems likely, litter size influences plasma E_2 levels in intact mice. Secondly, it seemed possible that, unlike the case in the rat, ovariectomy would induce placental overgrowth in the mouse only if the ovaries were removed earlier, at a time close to implantation. These possibilities were examined in the present study. A preliminary account of a portion of the results has appeared elsewhere (Miller 1979*a*).

Materials and Methods

Randomly bred female mice of the Quackenbush strain had their left ovaries or oviducts removed 3–4 weeks prior to mating. Thus after mating the mice became unilaterally pregnant. This enabled the collection of data for RNA and protein metabolism in the non-pregnant uterine horns of the same mice. The latter results formed part of a study of the systemic regulation of uterine growth during pregnancy (Miller 1979*b*). The results obtained for the placenta should not have been much influenced by the mice being unilaterally pregnant, since placental growth in bilaterally pregnant mice is not affected by the distribution of implantations between uterine horns (McLaren 1965).

Females were housed four to a box with a single fertile male (lights on between 0600 and 1800 h) and those with vaginal copulatory plugs were removed each morning and designated day 1 pregnant.

Experiment 1

In order to increase the variation in litter size, one-half of the mice had their left oviducts removed and the remainder were unilaterally ovariectomized prior to mating. Mice were laparotomized on day 10 and those that were pregnant were allocated at random to two groups. Mice in group 1 were sham-ovariectomized and received no further treatments. Those in group 2 were ovariectomized (the remaining right ovary or both ovaries, from mice which had lost their left ovaries or left oviducts before mating, respectively), and recieved daily s.c. injections of 1·0 mg P and 10 ng E_2 in 0·1 ml peanut oil on days 10–15. All animals were killed on day 16.

Experiment 2

All mice were unilaterally ovariectomized before mating. Mice were laparotomized on the afternoon of day 5 and any which did not exhibit small swellings along the right uterine horns corresponding to implantation sites were rejected. The remainder were allocated at random to six groups. Groups 1 and 4 were sham-ovariectomized on day 5 and received no further treatments. The remaining groups were ovariectomized at laparotomy on day 5 and received daily injections of 1·0 mg P and 10 ng E_2 (groups 2 and 5) or 1·0 mg P and 60 ng E_2 (groups 3 and 6) from day 5 onwards. Approximately one-half of the mice receiving each treatment was killed on day 10 (groups 1, 2 and 3), the remainder on day 16 (groups 4, 5 and 6).

In each experiment all mice received a single intravenous injection of 5·0 μCi [5-^{3}H]uridine (sp. act. 5 Ci/mmol, Radiochemical Centre, Amersham) in 0·2 ml 0·9% (w/v) NaCl solution 150 min before killing. Animals were killed by cervical dislocation, the uteri were promptly dissected and the numbers of live and dead foetuses were recorded. Estimates were made of the number of foetuses that were being resorbed or had aborted. The pregnant uterine horn was carefully slit lengthwise and live foetuses and their corresponding placentae were dissected, pooled, gently blotted and weighed. Estimates of mean placental protein, DNA and RNA : DNA ratio for each mouse were obtained

(Miller 1978), and the incorporation of [5-^{3}H]uridine into the RNA fraction was measured (Miller and Baggett 1972) and expressed as ^{3}H dpm per microgram placental DNA. The significance of differences between treatments and of the effects of litter size was determined by Student's *t*-test and the regression of each response against the number of live foetuses on day 16, respectively (Steel and Torrie 1960). It should be noted that when the results of these experiments are contrasted with data in the literature, days 5, 10 and 16 in the mouse are not equivalent to the same days of pregnancy in the rat. Parturition usually occurs late on day 19 or early on day 20 in bilaterally pregnant mice of this strain. It is suggested that in terms of embryonic and foetal development days 5, 10 and 16 in these mice correspond approximately to day 6, days 11–12 and days 18–20 respectively in the rat.

Results

Experiment 1: Effects of Litter Size on Placental Growth

A total of 59 mice were confirmed pregnant on day 10. Of these, four which were ovariectomized on day 10 contained no living foetuses on day 16. One of the four was observed aborting foetuses on day 14, and on day 16 it appeared that the foetuses in the other three mice had been lost at about the same time. The surgical treatments given prior to mating had a substantial effect ($P < 0{\cdot}001$) on the apparent ovulation rate in the right ovary, as estimated from the total numbers of live, dead, resorbing and aborted foetuses. The mean $\pm$ s.e.m. for the total in 24 mice which were unilaterally ovariectomized before mating was $11{\cdot}0 \pm 0{\cdot}7$, and in 31 mice from which the left oviduct was removed, $6{\cdot}8 \pm 0{\cdot}5$. Apart from these effects on ovulation in the right ovary, it seemed unlikely that the treatments given prior to mating influenced the subsequent function of the pregnant uterine horn and placentae, since all surgical procedures were confined to the left ovary and genital tract. Therefore, for any given litter size, the data from animals receiving different treatments prior to mating were pooled. Ovariectomy on day 10 had no overall effect on foetal survival or foetal and placental growth between days 10 and 16 of pregnancy. In 31 intact mice the means $\pm$ s.e.m. for live and total (live, dead, resorbed and aborted) foetuses, $7{\cdot}9 \pm 0{\cdot}7$ and $8{\cdot}5 \pm 0{\cdot}7$, and for foetal and placental weight were 382 ± 13 and $106{\cdot}4 \pm 3{\cdot}9$ mg, respectively, while in 24 mice ovariectomized on day 10 the corresponding means $\pm$ s.e.m. were $7{\cdot}2 \pm 0{\cdot}7$ and $8{\cdot}7 \pm 0{\cdot}7$, and 392 ± 14 and $112{\cdot}1 \pm 4{\cdot}0$ mg.

To reveal possible effects of litter size, the results (Table 1) are presented for three classes of mice, based on the number of live foetuses present on day 16. Changes in litter size did not affect foetal weight. Spontaneous placental hyperplasia due to low litter size occurred to an equal extent in intact and ovariectomized mice, as shown by the data and regression slopes (*b*) for placental weight and DNA. There was no effect of litter size on RNA : DNA ratio, or, curiously, on total protein. Within each of the three classes of mice ovariectomy on day 10 had no effect on placental weight, protein, DNA or RNA : DNA ratio. However, the highly significant effect of litter size on [5-^{3}H]uridine incorporation into placental RNA seen in intact mice disappeared when mice were ovariectomized on day 10. In order to test whether the regression slopes in intact and ovariectomized mice differed, the data for ^{3}H dpm per microgram DNA were transformed to $\log_{10}$, to equalize variance in the two treatment groups. The difference, $b_{\text{intact}} - b_{\text{ovx}}$, was significant ($0{\cdot}001 < P < 0{\cdot}01$).

Experiment 2: Effects of Ovariectomy on Day 5 and E_2 Dose on Placental Growth

The occurrence of implantation was confirmed in 119 mice laparotomized on day 5. Of these, seven in group 5 did not contain living foetuses on day 16, and the results for group 5 (Table 2) are for the remaining 17 mice which were pregnant on day 16.

In these 17 mice the mean number of live foetuses was reduced to 7·8, as compared to means of 9·7 and 10·2 in groups 4 and 6, respectively.

Table 1. Effects of litter size on foetal and placental growth in intact and ovariectomized mice

All mice were killed between 1200 and 1330 h on day 16 of pregnancy. Results are expressed as means ± s.e.m. for the numbers (*n*) of mice shown. Group 1 mice were intact (sham ovariectomy on day 10); group 2 mice were ovariectomized on day 10 and received 1·0 mg P and 10 ng E_2 daily on days 10–15

Litter size[A]	*n*	Foetus wet wt (mg)	Placenta				
			Wet wt (mg)	Protein (mg)	DNA (μg)	RNA : DNA	^{3}H dpm/ μg DNA
			Group 1				
1–5	7	373 ± 42	122 ± 13	7·34 ± 0·38	485 ± 29	1·76 ± 0·06	4·37 ± 0·53
6–9	12	411 ± 15	108 ± 5	8·31 ± 0·32	447 ± 18	1·71 ± 0·06	3·11 ± 0·23
10–14	12	359 ± 20	96 ± 4	8·11 ± 0·34	389 ± 11	1·70 ± 0·04	2·66 ± 0·22
b[B]		−3·85$^{n.s.}$	−3·74**	0·05$^{n.s.}$	−12·9***	−0·009$^{n.s.}$	−0·21***
			Group 2				
1–5	8	384 ± 16	123 ± 6	8·33 ± 0·33	511 ± 23	1·65 ± 0·05	5·94 ± 1·01
6–9	10	394 ± 16	114 ± 6	8·30 ± 0·50	451 ± 21	1·72 ± 0·04	6·02 ± 0·30
10–14	6	412 ± 46	93 ± 5	7·36 ± 0·38	376 ± 17	1·72 ± 0·05	5·52 ± 0·46
b[B]		−0·48$^{n.s.}$	−3·47**	−0·09$^{n.s.}$	−15·1**	0·010$^{n.s.}$	−0·03$^{n.s.}$

[A] Number of live foetuses on day 16.

[B] Slope of regression of response on litter size and level of significance at which slope differs from zero; *** $P < 0·001$, ** $0·001 < P < 0·01$, n.s. $P > 0·05$.

Table 2. Effects of ovariectomy at the time of implantation on foetal and placental growth

All mice were killed between 1200 and 1330 h on days 10 (groups 1, 2 and 3) or 16 (groups 4, 5 and 6) of pregnancy. Results are expressed as means ± s.e.m. for the numbers (*n*) of mice shown. Mice in groups 1 and 4 were intact (sham ovariectomy on day 5); mice in groups 2 and 5 were ovariectomized on day 5 and received 1·0 mg P and 10 ng E_2 daily from day 5 onwards; mice in groups 3 and 6 were also ovariectomized on day 5 and received 1·0 mg P and 60 ng E_2 daily from day 5 onwards

Group	*n*	Litter size[A]	Foetus wet wt (mg)	Placenta				
				Wet wt (mg)	Protein (mg)	DNA (μg)	RNA : DNA	^{3}H dpm/ μg DNA
1	16	11·6 ± 0·8	—	22·6 ± 0·7	1·68 ± 0·07	91 ± 3	2·08 ± 0·03	13·6 ± 1·6
2	20	11·5 ± 0·6	—	20·3 ± 0·7	1·56 ± 0·05	85 ± 3	2·06 ± 0·03	15·9 ± 1·5
3	19	11·3 ± 0·8	—	18·9 ± 0·6	1·52 ± 0·07	86 ± 3	2·14 ± 0·03	10·6 ± 1·0
4	16	9·7 ± 0·8	370 ± 15	114 ± 4	9·02 ± 0·30	405 ± 16	1·84 ± 0·04	2·94 ± 0·18
5	17	7·8 ± 1·2[B]	433 ± 12	123 ± 6	9·74 ± 0·59	443 ± 22	1·78 ± 0·04	5·85 ± 0·20
6	24	10·2 ± 0·6	403 ± 12	111 ± 4	8·47 ± 0·24	398 ± 10	1·79 ± 0·03	5·02 ± 0·22

[A] Number of live foetuses on day of killing.

[B] A further seven mice in group 5 had no live foetuses on day 16.

On day 10 the weight of the placenta was slightly reduced in ovariectomized mice (group 2 *v.* group 1, $0·01 < P < 0·5$; group 3 *v.* group 1, $P < 0·001$), but there was no effect of ovariectomy on placental protein, DNA, RNA : DNA ratio or [5-^{3}H]-uridine incorporation into RNA. Within the ovariectomized mice a sixfold increase in E_2 dose did not change placental weight, protein, DNA or RNA : DNA ratio on day 10, but did reduce the incorporation of [5-^{3}H]uridine (group 3 *v.* group 2, $0·001 < P < 0·01$). Foetal weights were not recorded on day 10.

On day 16 there was a small increase in foetal weight in ovariectomized mice receiving 10 ng E_2 daily as compared to control, intact mice (group 5 *v.* group 4, $0{\cdot}001 < P < 0{\cdot}01$). Ovariectomy on day 5 had no effect on placental weight, protein, DNA or RNA : DNA ratio on day 16, but did increase the incorporation of [5-^{3}H]uridine into placental RNA (groups 5 and 6 *v.* group 4, $P < 0{\cdot}001$). Within the ovariectomized mice increasing the dose of E_2 did not change placental weight or RNA : DNA ratio on day 16, but did slightly reduce placental protein, DNA and [5-^{3}H]uridine incorporation into RNA (group 6 *v.* group 5, $0{\cdot}01 < P < 0{\cdot}05$).

Discussion

The results of experiment 1 show that, regardless of litter size, placental overgrowth is not induced when mice are ovariectomized on day 10 and given P and a minimal dose of E_2 daily to maintain gestation. (A daily dose of 1·0 mg P alone will not maintain gestation in these mice—Miller 1978). It could be argued from the results of experiment 1 alone that the greater size of placentae in mice with small litters resulted primarily from an abnormal retardation of placental growth in mice with large litters, due to the non-physiological crowding of 10–14 live foetuses into one uterine horn. This was not the case, since the mean placental weights and amounts of DNA in mice with 10–14 foetuses were almost identical to those seen previously in bilaterally pregnant Quackenbush mice with a similar total number of foetuses (Miller 1978). Hence McLaren's (1965) finding that the effect of litter size on placental weight is entirely systemic in bilaterally pregnant mice can be extended to embrace unilaterally pregnant mice. The spontaneous placental overgrowth seen in mice with small number of foetuses evidently represents primarily hyperplasia, since it is associated with a substantial increase in DNA and little change in RNA : DNA ratio. The absence of an increase in placental protein in these mice was unexpected. Placental protein was correlated to placental weight in both intact ($r = 0{\cdot}438$, $0{\cdot}01 < P < 0{\cdot}025$) and ovariectomized ($r = 0{\cdot}646$, $P < 0{\cdot}001$) animals. The placental protein : DNA ratio may decrease with diminishing litter size, but perhaps an effect of litter size on placental tissue protein was masked in these mice by the presence in the placentae of considerable erythrocyte and plasma protein. Since the amount of spontaneous placental overgrowth in mice with small litters was not decreased after ovariectomy on day 10, it can be concluded either that oestrogens do not regulate placental growth in the mouse or that litter size does not influence plasma oestrogen levels in this species. Unfortunately no direct evidence is available for an effect of litter size on plasma oestrogen levels in the mouse, but such an effect seems likely, in view of the large effects of litter size on plasma E_2 concentration in the rat (Csapo and Wiest 1973).

The daily administration of 1·0 mg P and 10 ng E_2 to ovariectomized mice from day 5 onwards (expt 2) resulted in a less-than-optimal uterine environment for foetal development. Foetal development up to day 10 appeared normal and observations of abortions in some of these mice on days 14–15 suggest that the uterus tended to 'fail' at about the time that plasma E_2 levels begin to increase in intact mice (Barkley *et al.* 1977). Ovariectomy at the time of implantation and maintenance of gestation with 1·0 mg P and a minimal dose of E_2 also failed to elicit any significant placental overgrowth (group 5 *v.* group 4, expt 2). Within the ovariectomized mice a sixfold increase in the daily E_2 dose did appear to inhibit slightly placental growth by day 16. However, this apparent effect of E_2 dose was minor in comparison to equivalent

effects seen in ovariectomized rats (Csapo and Csapo 1973; Csapo and Wiest 1973) and may have been attributable to the difference between the numbers of live foetuses in mice in groups 5 and 6 rather than to E_2 dose *per se* (McLaren 1965). This interpretation is supported by the results of an earlier study, in which mice were ovariectomized on day 10 and given 1·0 mg P and one of a wide range of doses of E_2 (10, 25, 40, 160, 640 and 2560 ng) daily. In these the mean number of live foetuses per mouse was uniformly high (10·9–12·0) and placental growth up to day 16 was almost identical in all groups (Miller 1978). It is perhaps not surprising that similar results were obtained following ovariectomy on days 5 and 10, as most of the growth of the placentae occurs between days 10 and 16 (at which time the placenta attains its maximal weight—Miller 1978). The data for the effects of ovariectomy on day 5 eliminate the possibility that placental growth in the mouse in the latter half of gestation is programmed by circulating oestrogen levels prior to day 10.

The physiological significance of ovariectomy-induced placental hyperplasia in the rat remains unknown. It does not result in increased placental progesterone synthesis (Chan and Leathem 1977), and the rat placenta cannot synthesize oestrogens (Sybulski 1969). Finn and Porter (1975) have reviewed some of the data for endocrine regulation of placental growth in the rat. They concluded that since diminished plasma oestrogen levels cannot induce placental oestrogen synthesis and probably do reduce blood flow through the uterus, ovariectomy-induced placental hyperplasia may represent a response which enhances the exchange of nutrients, etc. between the foetus and maternal circulation in the presence of a diminished uterine blood flow. The striking effect of ovariectomy on the incorporation of [5-^{3}H]uridine into placental RNA in 'normal litter' ($\geqslant$ six foetuses) mice seen in the present experiments cannot be explained without further studies. The difference in incorporation after giving a single intravenous pulse of [5-^{3}H]uridine on day 16 presumably does not indicate any substantial effect of ovariectomy on RNA synthesis, since the amounts of RNA in the two groups of placentae on day 16 were very similar. Blood flow to the uterus and placentae was not determined in these experiments. Nevertheless, it seems likely that the data for [5-^{3}H]uridine incorporation reflect differences in blood flow to the uterus and/or placentae. In 'normal litter' mice the higher incorporation of [5-^{3}H]uridine in ovariectomized mice could reflect enhanced blood flow to the placentae, but it could equally reflect a diminished transfer of the nucleoside from the placenta to the foetus after ovariectomy. Whatever the true significance of these data may be, the results indicate that if any change in placental blood flow and/or exchange occurs in response to ovariectomy and low plasma E_2 levels, the adaptation does not require placental hyperplasia.

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