Can reduced consumption of gonadotrophins account for ovarian compensation in unilaterally ovariectomized, immature mice injected with gonadotrophins?

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Summary. The effect of unilateral ovariectomy on ovulation rates in immature mice was studied. Ovulations were induced by injecting PMSG and hCG and their number was determined by counting tubal oocytes. A 2–3-fold increase in number of ovulations per ovary was observed after unilateral ovariectomy, and daily injections of progesterone abolished this ovulatory compensation. No significant increase in serum concentrations of immunoreactive FSH and LH was observed at 4, 8, 32 and 51 h after unilateral ovariectomy. Progesterone treatment lowered FSH levels at all times, while LH was unaffected. In intact mice, ovarian sensitivity to PMSG and hCG was not substantially affected by progesterone. Ovulatory compensation in immature gonadotrophin-injected mice appears to arise through a negative feedback mechanism and transiently increased secretion of pituitary gonadotrophin rather than through a greater utilization of a fixed amount of gonadotrophin.

Introduction

If one ovary is removed from a mouse, the remaining ovary compensates by ovulating about twice as many oocytes in subsequent cycles. This response is usually attributed to increased secretion of pituitary gonadotrophins (GTH), provoked by a reduction in the concentration of ovarian hormones in blood—an example of the well-established negative feedback relationship that exists between ovarian hormone secretion and hypothalamus–pituitary hormone secretion. A contending view is that compensation arises because the single remaining ovary has access to a greater proportion of a limited supply of pituitary GTH when consumption of GTH by its partner is eliminated (for review see Bruzzone, Lipschutz & Niedmann, 1951). This view has not been ruled out (Welschen, 1970; Labhsetwar, 1973; Peters & Braathen, 1973; Ying & Gove, 1973) and indeed seems to be strongly supported by the observations of McLaren (1966) that injected GTH produces twice as many ovulations by an ovary of an adult or immature 26-day-old mouse if its partner is absent.

This view of McLaren's experiment requires the assumption that any endogenous GTH could be neglected and that the total GTH present was that in the injections. This assumption is vulnerable since increases in GTH secretion may have occurred after unilateral ovariectomy. Such increases could add their effects to those of the injected GTH, or could influence the sensitivity of the ovary, and increase the number of ovulations.

We set out to reproduce (in immature mice) the most striking of McLaren's observations which challenge the conventional feedback hypothesis. In addition, the experiments were repeated in mice injected with progesterone in an attempt to eliminate, by feedback action, any rise in endogenous GTH secretion. These were designed to help show whether the feedback hypothesis can withstand the challenge and remain the best single explanation of ovarian

compensation, even when exogenous GTH is used in immature mice. In addition, plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by radioimmunoassay (RIA).

Materials and Methods

Immature female Swiss mice, weighing 8–15 g, were housed with 12 h light daily and with free access to standard food (GR 2 + pellets; Clark King) and water. When they were 22–24 days old, either the right or the left ovary, chosen randomly, was removed under tribromoethanol anaesthesia (Avertin: Winthrop Laboratories; 0.01 ml/g intraperitoneally (i.p.)). Control mice were sham-operated, i.e. they were anaesthetized, their skin and body wall were incised and the incisions were sutured. These operations were carried out between 08:00 and 10:00 h (see Text-fig. 1).

Text-fig. 1. Schedule of the experiments to show the times of unilateral ovariectomy, gonadotrophin injection and death. Mice that were treated with progesterone (not shown) were injected at 14:00–15:00 h on Days 0, 1, 2 and 3.

Ovulations were induced by injection of GTH, closely following McLaren’s (1966) procedure. On the day of unilateral ovariectomy (Day 1) 0.5–2 i.u. PMSG (Primantrol: Schering A.G.) was injected i.p. between 16:30 and 17:30 h. At noon on Day 3, 43 h later, 5 i.u. hCG (Pregnyl: Organon Laboratories Ltd) was injected i.p. All injections were made in a volume of 80–100 µl of a 1% (w/v) solution of human serum albumin (Commonwealth Serum Laboratories, Melbourne) in distilled water. The mice were killed 20 to 21 h after hCG injection (08:00–09:00 h on Day 4). Using a binocular microscope, the distended ampulla of the oviduct was located and its wall cut. The ovulated oocytes in their cumulus mass were gently expressed through the cut into a Petri dish containing physiological saline (9 g NaCl/l), transferred to a microscope slide and counted under low power (×45 magnification).

Sensitivity to PMSG differs among strains of mice (Lamond, 1960; Falconer, Edwards, Fowler & Roberts, 1961). The doses of 0.5 to 2 i.u. were chosen for the present experiments because, in preliminary observations on intact mice, these doses were on the rising part of the dose-response relationship (Table 1) and produced similar numbers of ovulations to those found by McLaren (1966). For these doses histological examination of the ovaries revealed no luteal bodies with entrapped oocytes, whereas for high doses (above 8 i.u.) these bodies occurred in increasing numbers and numbers of ovulations declined (Table 1), as has been reported by Wilson & Zarrow (1962).

Table 1. Dose–response relationship for the number of ovulations (mean ± s.e.m. per ovary) in intact, immature mice (no. in parentheses) injected with various doses of PMSG followed by 5 i.u. hCG

	Dose of PMSG (i.u.)						
	0.5	1	2	4	8	16	32
No. of oocytes per ovary	3.4 ± 0.3 (12)	3.6 ± 0.2 (16)	8.3 ± 1.0 (16)	20.5 ± 2.5 (4)	29.8 ± 1.4 (8)	17.3 ± 1.0 (6)	4.3 ± 1.8 (6)
							0.2 ± 0.2 (6)

In order to check the reliability of counts of tubal oocytes as an indicator of the number of ovulations, the counts were compared with numbers of corpora lutea (CL) in the corresponding ovaries. To do this, serial sections (14 μ m thick) of some ovaries were prepared using conventional histological methods. By examining every 3rd stained section it was possible to count genuine CL and confidently to distinguish them from luteal bodies containing trapped oocytes (Long & Evans, 1922). In preliminary experiments, 25 ovaries and their respective oviducts from 8 intact and 9 unilaterally ovariectomized mice were examined. There was good agreement between the counts of shed oocytes and corpora lutea over the ovulation ranges of 2–18 for intact and 3–13 for the ovariectomized mice: in 24 of the 25 counts the numbers were equal and in the other there were 5 CL but only 4 oocytes.

Progesterone (Sigma Chemical Co.) was dissolved in ethanol:peanut oil (1:9 v/v) for subcutaneous (s.c.) injection of 1 mg in 0.05 ml vehicle. Injections were given daily at 14:00–15:00 h, beginning the day before unilateral ovariectomy (Day 0), until the mice were killed. Corresponding control animals were injected s.c. with 0.05 ml vehicle. In all these mice unilateral ovariectomies and gonadotrophin treatment were carried out as described above. To check that treatment with progesterone did not affect accuracy of oocyte counting, counts of corpora lutea were also carried out on the ovaries of 6 intact and 9 unilaterally ovariectomized mice that were treated with progesterone and induced to ovulate by injecting PMSG and hCG. Counts ranged from 0 to 6 in all the mice, and for all the oocyte count was equal to the CL count in the corresponding ovary.

Blood samples pooled from groups of 5 (occasionally 4, 6 or 7) mice were used for FSH and LH estimation. The samples, collected from the neck vessels severed during decapitation, were allowed to clot and the serum, obtained after centrifugation, was stored at -15°C until assayed.

Mouse LH was measured by RIA using reagents for the rat LH system provided by the National Institute of Arthritis, Metabolic and Digestive Diseases (NIAMDD), NIH, Bethesda, Maryland. The characteristics of this assay as used by us have been described previously (Lee, de Kretser, Hudson & Wang, 1975) and it has been used by other investigators to measure mouse LH (Kovacic & Parlow, 1972). The sensitivity was 0.11 ± 0.06 (s.d.) ng and the within-assay precision was 3.2 to 8.7%. Mouse FSH was measured by a double-antibody system, also using reagents supplied by NIAMDD except that the first antibody (59 No. 3) was raised by one of us (V.W.K.L.) against human FSH (LER-1563). The characteristics of the assay are shown in Text-fig. 2. Sensitivity ranged from 3 to 5.5 ng/tube and the within-assay precision from 2.9 to 7.0% at the optimal part of the curves. PMSG at 0.5 i.u./ml, a concentration higher than could occur in the serum samples, did not cross-react with antibodies to either FSH or LH.

The statistical significance of differences between treatment groups was determined using two-tailed, unpaired *t* tests.

Results

Ovulatory compensation after unilateral ovariectomy

In 3 experiments, using different doses of PMSG, injection of PMSG and hCG consistently induced more ovulations per ovary in the experimental mice than in sham-operated controls (Table 2). When the dose of PMSG was 0.5 i.u. the increase was more than 3-fold (ranges: 0–3.5 in control, 6–10 in experimental mice; $P < 0.001$), with 1 i.u. it was more than 2-fold (ranges: 3–4.5 in control, 7–11 in experimental mice; $P < 0.001$) and with 2 i.u. it was 48% (ranges: 5–10 in control, 10–15 in experimental mice; $P < 0.01$).

Inhibition of ovulatory compensation by progesterone

The experiments on ovulatory compensation after unilateral ovariectomy were repeated, but with the addition of experimental groups injected daily with progesterone in an attempt to

Text-fig. 2. Radioimmunoassay of FSH in mouse serum using the antiserum 59 No. 3 at 1:10 000 dilution. Parallel dose-response curves for pituitary FSH and serum samples are shown for both rat and mouse. Lack of cross-reaction by rat or mouse LH or by α - or β -subunits of rat LH is also shown.

Table 2. Effect of unilateral ovariectomy on mean $\pm$ s.e.m. numbers of tubal oocytes counted (per ovary) in mice (8/group) injected with 0.5, 1 or 2 i.u. PMSG followed by 5 i.u. hCG.

	Dose of PMSG (i.u.)		
	0.5	1	2
Sham-operated	2.6 $\pm$ 0.6	3.6 $\pm$ 0.2	8.3 $\pm$ 1.1
Unilaterally ovariectomized	8.3 $\pm$ 0.5***	7.9 $\pm$ 0.8***	12.4 $\pm$ 0.5**

** $P < 0.01$; *** $P < 0.001$; differences from sham-operated controls.

suppress secretion of GTH. There were then four groups: (1) sham-operated, no progesterone; (2) unilaterally ovariectomized, no progesterone; (3) sham-operated, progesterone-injected; and (4) unilaterally ovariectomized, progesterone-injected.

Two experiments were carried out: in Exp. A the dose of PMSG was 0.5 i.u. and in Exp. B 1 i.u. was used. In Exp. A the ovariectomized mice (Group 2) shed 2–3 times as many oocytes as

did sham-operated mice (Group 1) if no progesterone was given (Table 3). But when progesterone was injected there was no compensation; the number of ovulations in the Group 4 mice was significantly lower ($P < 0.001$) than that in Group 2 mice and was not significantly different from that in Group 3. A similar result occurred in Exp. B (Table 3). The reduction in magnitude of the compensatory response in the progesterone-treated mice was highly significant ($P < 0.001$) as shown by the Wilcoxon one-sample test (Lehmann, 1975) adapted to test for interaction between progesterone treatment and unilateral ovariectomy.

Table 3. Effect of progesterone (1 mg daily) and of unilateral ovariectomy on mean $\pm$ s.e.m. numbers of tubal oocytes (per ovary) in mice (no. in parentheses) injected with 0.5 or 1 i.u. PMSG followed by 5 i.u. hCG

Group	Experiment A (0.5 i.u. PMSG)	Experiment B (1 i.u. PMSG)
1 Sham-operated, no progesterone	3.3 ± 0.9 (7)	4.7 ± 0.3 (10)
3 Sham-operated, progesterone	2.8 ± 0.7 (7)	4.0 ± 0.1 (10)
2 Unilateral ovariectomy, no progesterone	$*7.6 \pm 0.2$ (7)	$*9.3 \pm 0.7$ (10)
4 Unilateral ovariectomy, progesterone	$\dagger 4.3 \pm 0.6$ (7)	$\dagger 3.4 \pm 0.6$ (9)

* $P < 0.001$; difference from Group 1.

† $P < 0.001$; difference from Group 2.

In both experiments progesterone had little effect on the numbers of ovulations produced by GTH in the intact mice. In Exp. A there was no significant difference and in Exp. B there was a reduction ($P < 0.05$) of fewer than one oocyte per ovary.

Serum FSH and LH

Serum concentrations of FSH and LH were measured at various times after surgery in sham-operated and unilaterally ovariectomized mice. These mice were of the same age (22–24 days) and underwent the same surgical and hormonal treatment as for the experiments described above. Blood samples were taken at the following times (see Text-fig. 1 for their relationship to experimental time course): 09:00 h on Day 1, 13:00 h on Day 1, 17:00 h on Day 1, 17:00 h on Day 2 and 12:00 h on Day 3.

Before unilateral ovariectomy (09:00 h on Day 1) the mean FSH concentration in serum was 174 ng/ml. At all later times there were low levels (≤ 62 ng/ml) which were not increased by the ovariectomy (Table 4). Progesterone treatment reduced ($P < 0.01$) FSH levels to 87 ng/ml at the time of surgery. After surgery, reductions in FSH levels due to progesterone treatment were significant ($P < 0.05$; data pooled from experimental and sham-operated groups) at all times except 17:00 h on Day 1.

Data for serum LH concentration, presented in Table 4, are less extensive than those for FSH because priority was given to FSH measurement when there was insufficient serum for both. None of the differences in LH levels of unilaterally ovariectomized or of progesterone-treated mice was significant at the 0.05 level.

FSH and LH were also measured in serum of mice that were bilaterally ovariectomized or bilaterally sham-operated (Table 5). These mice were not given PMSG and hCG but were otherwise treated in the same way as the unilaterally ovariectomized animals. In bilaterally ovariectomized mice, FSH levels were increased ($P < 0.01$) substantially at 17:00 h on Day 2 and at 12:00 h on Day 3. No changes were detected in LH levels.

Table 4. Serum concentrations of FSH and LH after unilateral ovariectomy (ULO) and progesterone treatment (1 mg/day)

Time of sampling (h after surgery)	FSH (ng NIAMD-rat-FSH-RP1/ml)				LH (ng NIAMD-rat-LH-I3)			
	No progesterone		Progesterone		No progesterone		Progesterone	
	Control	ULO	Control	ULO	Control	ULO	Control	ULO
09:00 h, Day 1 (0)	*174 ± 11 (5)		*87 ± 20 (5)		0.40 ± 0.04 (4)		0.28 ± 0.04 (5)	
13:00 h, Day 1 (4)	45 ± 3 (8)	49 ± 3 (7)	40 ± 3 (6)	32 ± 5 (6)	0.24 ± 0.07 (8)	0.28 ± 0.06 (4)	0.28 ± 0.04 (5)	0.35 ± 0.08 (7)
17:00 h, Day 1 (8)	45 ± 5 (6)	57 ± 16 (5)	39 ± 4 (7)	39 ± 10 (5)	0.23 ± 0.18 (2)	0.3 (1)	2.02 ± 1.23 (6)	0.44 ± 0.07 (5)
17:00 h, Day 2 (32)	62 ± 5 (5)	55 ± 3 (5)	53 ± 1 (4)	47 ± 5 (4)	0.87 ± 0.15 (3)	—	0.90 ± 0.20 (3)	0.15 ± 0.05 (2)
12:00 h, Day 3 (51)	52 ± 4 (4)	42 ± 4 (3)	37 ± 6 (5)	36 ± 1 (4)	2.30 ± 0.38 (4)	2.57 ± 0.32 (3)	2.52 ± 0.34 (5)	1.70 ± 0.34 (4)

All mice sampled on Days 2 and 3 had been injected with 1 i.u. PMSG at 17:00 h on Day 1. Control mice were sham-operated. Values are mean ± s.e.m., the no. in parentheses represents the number of serum samples, each sample being pooled from a group of 5 mice. * These values were significantly different, *P* < 0.01.

Table 5. Serum concentrations of FSH and LH (see Table 4) after bilateral ovariectomy of mice

Time of sampling (h after surgery)	FSH (ng/ml)		LH (ng/ml)	
	Control	Bilateral ovariectomy	Control	Bilateral ovariectomy
13:00 h, Day 1 (4)	74 ± 9 (4)	97 ± 16 (4)	0.27 ± 0.07 (3)	0.50 ± 0.30 (2)
17:00 h, Day 1 (8)	80 ± 14 (4)	132 ± 26 (4)	0.35 ± 0.05 (2)	0.73 ± 0.23 (3)
17:00 h, Day 2 (32)	57 ± 7 (7)	*211 ± 34 (9)	0.62 ± 0.12 (6)	0.38 ± 0.06 (6)
12:00 h, Day 3 (51)	45 ± 4 (4)	*349 ± 91 (2)	0.30 ± 0.07 (4)	0.50 ± 0.10 (2)

Values are mean ± s.e.m., the no. in parentheses representing the number of serum samples, each sample being pooled from a group of 5 mice.

* These values were significantly different from the corresponding control group, $P < 0.01$.

Discussion

The results fully confirmed the finding by McLaren (1966) of an enhancement by unilateral ovariectomy of the ovulatory response to injection of sub-maximal doses of PMSG. In seeking to establish whether the enhancement requires an increased contribution by the mouse's own pituitary gland, two approaches have been used; firstly, blockade of any rise in secretion by injecting progesterone and, secondly, measurement of circulating endogenous FSH and LH.

The fact that progesterone treatment prevented the enhancement induced by unilateral ovariectomy suggests that the compensation after this operation is caused by increased pituitary secretion of GTH rather than by the GTH-sparing effect proposed by McLaren (1966). It can be argued, in defence of a consumption mechanism, that the progesterone simply reduced ovarian sensitivity to the injected GTH but this seems unlikely since any reduction of ovulations in the sham-operated mice was slight and may be attributable to the demonstrated suppression of endogenous FSH. In contrast, progesterone treatment in unilaterally ovariectomized mice so reduced the number of ovulations that no compensation was evident. Oestrogens were not used in the attempt to block pituitary GTH secretion because they directly stimulate ovarian follicles and synergize with GTH (Goldenberg, Vaitukaitis & Ross, 1972).

Numbers of ovulations were estimated by an indirect method (counting tubal oocytes) so that caution was required because of the possibility that the experimental treatments, especially progesterone, could lead to miscounts and invalidate the method, e.g. through altering oviductal motility or altering the time of ovulation (Zarrow & Gallo, 1969). Reassurance on this point was given by the near-perfect agreement between oocyte counts and counts of corpora lutea in all the experimental treatments.

The present experiments using progesterone treatment to nullify any feedback mechanism support earlier attempts, using hypophysectomy, to show that ovulatory compensation arises through a feedback mechanism rather than a consumption mechanism. In three experiments on hypophysectomized rats, PMSG and hCG produced no more ovarian response (weight, follicular development or ovulations) in unilaterally ovariectomized rats than in those with intact ovaries (Selye, 1940; Greenwald, 1968; Welschen, 1972). The hypophysectomy experiments alone are not conclusive since hypophysectomy reduces ovarian sensitivity to PMSG (Williams, 1944) and one cannot judge whether the doses of PMSG were at the critical levels required to show ovulatory compensation (Zarrow, Sundaram & Stob, 1965; McLaren, 1966).

An additional possible mechanism is that the responsiveness of the remaining ovary of unilaterally ovariectomized animals to given amounts of GTH is somehow enhanced by the absence of the other ovary, either through a possible neural mechanism (e.g. see Gerendai & Halász, 1978) or through the loss of secretions of the excised ovary. This mechanism cannot be ruled out either by the hypophysectomy experiments or by our experiment.

When measuring endogenous GTH, most attention was given to FSH, rather than to LH, because of the importance of FSH in developing follicles to ovulatory size and because hCG was given to the mice at a dose high enough to cause ovulation of the maximum number of fully developed follicles (Wilson & Zarrow, 1962). The decline in FSH levels seen after the first sampling time (09:00 h on Day 1) was striking, although not of special interest in relation to the objectives of the experiment. It may have been caused by the anaesthetic given to mice sampled at 13:00 h and later, or by mechanical stimulation of the ovarian pedicle which could elicit a neurally-mediated suppression (Burden, 1978). At this age (22–24 days) FSH levels are likely to be declining anyway (Stiff, Bronson & Stetson, 1974; Dullaart, Kent & Ryle, 1975), but presumably not at the rate suggested by our data for Day 1 (Table 4).

Progesterone was injected in an attempt to reduce GTH (especially FSH) secretion. It was clearly effective in reducing blood FSH, although there were still measurable concentrations, as would be expected from other reported measurements of circulating FSH, and observations of healthy, tertiary, ovarian follicles during chronic progesterone treatment (for references see Labhsetwar, 1969). The progesterone also would be expected to prevent any rise in GTH caused by removal of ovaries (Jelinek, Šeda & Marhan, 1968; Labhsetwar, 1969) but the present experiments provide no direct evidence on this question since, at the times sampled, we did not detect any increased GTH levels after unilateral ovariectomy.

The failure to show changes in plasma FSH and LH after unilateral ovariectomy in immature mice is not altogether surprising. Even in adult animals, including rats (Howland & Skinner, 1973), sheep (Findlay & Cumming, 1977) and hamsters (Bast & Greenwald, 1977), any early rises due to the ovariectomy appear to be transient. They may be erratic and it may be necessary to sample each animal serially to detect them, since secretion of GTH is episodic in some circumstances (Yen, Tsai, Vandenberg & Rebar, 1972; Knobil, 1974; Foster, Lemons, Jaffe & Niswender, 1975). Any failure to detect changes must be regarded only as provisional evidence that changes do not occur, but we should emphasize that each point in Table 4 is derived from a large number of mice (usually 25 or more). This represents a substantial attempt to detect any changes that occur at the selected times and, naturally, has led to fairly precise estimates of mean levels (small s.e.m.) even if values in individual mice do vary substantially (partly due to episodic secretion). The data are incompatible with large increases having occurred after unilateral ovariectomy. The RIA was clearly able to detect changes, at least of FSH, since a prompt rise in FSH was seen after bilateral ovariectomy.

Although FSH and LH measurement is clearly worthwhile when examining the mechanism of ovulatory compensation, such measurements cannot, on their own, be decisive about the two major hypotheses (feedback or consumption) concerning the mechanism of ovulatory compensation. Although McLaren (1966) associated the consumption hypothesis with unchanging levels of GTH, it seems that, even under that hypothesis, removal of an ovary would be expected to raise plasma GTH levels. In the present experiments, the absence of large changes in FSH or LH concentrations shows that any rises occurred only at times other than those sampled, or that neither the consumption hypothesis nor the feedback hypothesis is adequate to explain compensation.

In conclusion, the experiments have confirmed the striking observation (McLaren, 1966) that ovulatory compensation occurs after unilateral ovariectomy in immature mice induced to ovulate by exogenous gonadotrophins and, in addition, they provide support for the view that a feedback mechanism, rather than a consumption mechanism, may explain it. The present experiments, like those involving hypophysectomy, cannot be conclusive about the mechanism but they do provide independent support, resting on the observation that progesterone obliterated compensation, while not substantially reducing ovarian sensitivity to GTH action. Paradoxically, no rises in serum concentrations of FSH and LH were detected after unilateral ovariectomy. Resolution of the problem may be achieved by other means of examining the role of endogenous FSH and LH, e.g. by neutralization of these hormones with antibodies. However,

the difficulties encountered with both the feedback hypothesis and the consumption hypothesis do prompt renewed interest in possible neural mechanisms (Aron, Marx & Marescaux, 1948; Gerendai & Halász, 1978) that have generally been excluded from consideration but may have to be invoked for a final understanding of ovulatory compensation after unilateral ovariectomy.

Mouse pituitary GTH were kindly provided by Dr A. F. Parlow. The work of V.W.K.L. is supported by the Ford Foundation and by the National Health and Medical Research Council of Australia. We thank Ms Val Ford and Ms Julie McMaster for technical assistance, Dr A. Padmanabhan for his advice on adapting the Wilcoxon one-sample test to test interactions, and Dr I. R. McDonald for his helpful comments on the manuscript.

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Received 7 February 1979