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Ovulation Rate and Inhibin Levels in Gonadotrophin-treated Mice

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Abstract

Ovarian follicular growth was induced in immature female mice with varying doses of pregnant mare's serum gonadotrophin. The numbers of ovulations were determined either by counting tubal oocytes or corpora lutea in the ovary. Ovarian and circulatory levels of inhibin rose progressively with increasing doses of PMSG and a positive correlation ($P < 0{\cdot}01$) was found between circulating inhibin levels and ovulation rate. The latter correlation makes it likely that the growing preovulatory ovarian follicles are the predominant source for the secretion of inhibin into the circulatory system.

Introduction

In immature female rats, treatment with pregnant mare's serum gonadotrophin (PMSG) stimulated ovarian follicular growth and raised ovarian and circulatory levels of inhibin (Lee *et al.* 1981, 1982; Lee and Findlay 1982). The ovaries are the source of circulating inhibin since ovariectomy of PMSG-primed rats caused rapid disappearance of inhibin in peripheral blood (Lee *et al.* 1982). Furthermore, in rats that had been ovariectomized prior to PMSG administration, there was no increase in circulatory levels of inhibin (Lee *et al.* 1982). Also, compensatory ovarian secretion of inhibin was demonstrable in PMSG-primed rats after unilateral ovariectomy (Lee *et al.* 1982).

It is well known that superovulation occurs after PMSG treatment in rodents (Wilson and Zarrow 1962). Since inhibin levels in immature rats rise progressively as ovarian follicular growth is stimulated by increasing doses of PMSG it seems that the secretion of inhibin into the circulation may occur predominantly from preovulatory follicles. In this investigation we have, therefore, examined the relationship between ovulation rate and inhibin levels in the ovary and blood of immature mice.

Materials and Methods

Immature female Swiss mice were supplied by Monash University Central Animal House and used, in two experiments, when 21–24 days of age.

Experiment 1

Groups of mice were injected intraperitoneally (i.p.) at 1700 h with 0·2 ml of a 1% (w/v) solution of human serum albumin in distilled water containing either 0, 0·5, 2, or 8 i.u. of PMSG (Primantron, Schering AG, Sydney). At 24 and 48 h after injection of PMSG, subgroups of mice were decapitated and the blood collected. Blood samples were allowed to clot, centrifuged, and the serum samples stored at −15°C until

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assayed. Each serum sample was obtained from blood pooled from three mice. Ovaries and uteri were also dissected out and weighed, and the ovaries from the same subgroups of mice were similarly pooled for subsequent analysis.

Additional subgroups of the mice given 0·5, 2 or 8 i.u. of PMSG were injected i.p. 43 h later with 10 i.u. of human chorionic gonadotrophin (hCG; Pregnyl, Organon, Sydney), a dose high enough to induce the ovulation of the maximum number of follicles stimulated by PMSG (Wilson and Zarrow 1962). The mice were killed 23 h after hCG injection and the numbers of ovulations determined either by counting tubal oocytes or by counting corpora lutea seen in serial histological sections of the ovaries using procedures previously described (Gibson *et al.* 1979).

Experiment 2

The protocol used was essentially that described for experiment 1 but blood samples were collected only from subgroups of mice killed at 48 h after PMSG injection. A different dose range of PMSG (0·5, 1·5, 4·5 and 13·5 i.u.) was chosen in order to span the ascending part of the dose-response relationship for numbers of ovulations and with the top dose high enough to produce corpora lutea with entrapped oocytes rather than genuine ovulations. Other subgroups of mice were treated with PMSG followed by hCG 43 h later, and ovulation rate determined as described above.

Assays for Inhibin and FSH

Inhibin activity in sera and ovarian cytosols was measured using an *in vitro* pituitary cell culture and expressed in terms of an ovine testicular lymph protein preparation, designated to have an arbitrary potency of 1 U/mg (Eddie *et al.* 1979). The inhibin bioassay was based on inhibition of pituitary FSH cell content, and all samples were assayed for inhibin activity using at least two dilutions in triplicate culture wells. All samples within a single experiment were assayed in a single bioassay (index of precision, λ, was always less than 0·2) and the samples were prepared as described previously (Lee *et al.* 1981). Briefly, all samples were extracted with dextran-coated charcoal (1 mg/ml final dilution) to remove any steroids; the sera samples were additionally treated with 5% (w/v) polyethylene glycol to remove any cytotoxic factors. FSH was measured by radioimmunoassay (Gibson *et al.* 1979) in the $<10\%$ coefficient of variation region of the standard curve.

Statistical Analysis of Data

The data were analysed using analysis of variance with Duncan's new multiple range test and Pearson's correlation coefficient (Steel and Torrie 1980).

Results

The results of experiment 1 are summarized in Table 1. At 24 or 48 h after PMSG treatment, ovarian weight was significantly increased only by the highest dose of PMSG (8 i.u.). In contrast, uterine weight was elevated by all doses of PMSG at both times, with a dose-related increase observed by 48 h. The changes in ovarian and blood inhibin concentrations resembled those in ovarian weight, with significantly elevated levels observed only at the highest dose of PMSG. Inhibin levels in the ovary were correlated with inhibin levels in the blood ($r = 0{\cdot}54$, $n = 34$, $P < 0{\cdot}001$) and were negatively correlated with FSH concentrations in peripheral blood ($r = -0{\cdot}35$, $n = 35$, $P < 0{\cdot}05$). Circulatory levels of FSH were at all times significantly reduced in PMSG-treated mice. Ovulation rate was at least threefold higher with the highest dose of PMSG (8 i.u.) than with the other two doses. At 48 h after PMSG injection, both blood and ovarian inhibin levels were also increased approximately threefold.

In order to check the relationship observed between ovulation rate and inhibin levels, the experiment was repeated using a range of PMSG doses chosen to span the dose–response relationship of PMSG dose with ovulation rate. The results are illustrated in Fig. 1. At 48 h after PMSG treatment, circulatory concentrations of inhibin increased with increasing dose of PMSG ($F_{4,16} = 9{\cdot}75$, $P < 0{\cdot}001$) from $4{\cdot}4 \pm 0{\cdot}8$ U/ml (mean ± s.e.) in control mice to maximal levels of $44{\cdot}1 \pm 9{\cdot}0$ U/ml in mice injected

Table 1. Experiment 1: effect of PMSG on ovarian and uterine weights, ovarian and blood inhibin and FSH levels, and ovulation rate in immature female mice

Values for ovarian weight and concentrations of inhibin and FSH are mean ± s.e. based on samples pooled from three mice (n = 5–11 samples, usually 8 or 9). Values for uterine weight (n = 23–26) and numbers of oocytes (n = 15–17) are mean ± s.e. for individual mice. Within each time after PMSG treatment, mean values with different letter superscripts are significantly different ($P < 0{\cdot}05$) by Duncan's new multiple range test

Parameter	Time after PMSG (h)	Dose of PMSG (i.u.) 0	0·5	2	8
Ovarian weight (mg/ovary)	24	$1{\cdot}41 \pm 0{\cdot}09^{a}$	$1{\cdot}47 \pm 0{\cdot}05^{a}$	$1{\cdot}60 \pm 0{\cdot}07^{a}$	$2{\cdot}18 \pm 0{\cdot}16^{b}$
	48	$1{\cdot}45 \pm 0{\cdot}07^{a}$	$1{\cdot}60 \pm 0{\cdot}11^{a}$	$1{\cdot}65 \pm 0{\cdot}09^{a}$	$2{\cdot}64 \pm 0{\cdot}18^{b}$
Uterine weight (mg/animal)	24	$12{\cdot}8 \pm 0{\cdot}5^{a}$	$28{\cdot}0 \pm 2{\cdot}2^{b}$	$36{\cdot}0 \pm 2{\cdot}2^{c}$	$32{\cdot}7 \pm 1{\cdot}8^{bc}$
	48	$12{\cdot}7 \pm 0{\cdot}6^{a}$	$47{\cdot}5 \pm 3{\cdot}4^{b}$	$63{\cdot}6 \pm 4{\cdot}6^{c}$	$75{\cdot}2 \pm 4{\cdot}1^{d}$
Ovarian inhibin (U/ovary)	24	$4{\cdot}1 \pm 1{\cdot}1^{a}$	$3{\cdot}7 \pm 0{\cdot}3^{a}$	$4{\cdot}7 \pm 0{\cdot}8^{a}$	$7{\cdot}4 \pm 0{\cdot}8^{b}$
	48	$4{\cdot}1 \pm 0{\cdot}5^{a}$	$4{\cdot}9 \pm 0{\cdot}8^{a}$	$6{\cdot}5 \pm 1{\cdot}9^{a}$	$20{\cdot}2 \pm 3{\cdot}4^{b}$
Ovarian inhibin (U/mg)	24	$2{\cdot}95 \pm 0{\cdot}73^{a}$	$2{\cdot}59 \pm 0{\cdot}26^{a}$	$2{\cdot}93 \pm 0{\cdot}43^{a}$	$3{\cdot}57 \pm 0{\cdot}45^{a}$
	48	$2{\cdot}87 \pm 0{\cdot}37^{a}$	$3{\cdot}21 \pm 0{\cdot}53^{a}$	$3{\cdot}96 \pm 1{\cdot}11^{a}$	$8{\cdot}30 \pm 1{\cdot}67^{b}$
Blood inhibin (U/ml)	24	$13{\cdot}6 \pm 2{\cdot}1^{a}$	$12{\cdot}4 \pm 3{\cdot}1^{a}$	$14{\cdot}7 \pm 3{\cdot}0^{a}$	$28{\cdot}9 \pm 5{\cdot}8^{b}$
	48	$11{\cdot}4 \pm 1{\cdot}2^{a}$	$20{\cdot}0 \pm 2{\cdot}1^{a}$	$18{\cdot}6 \pm 1{\cdot}3^{a}$	$68{\cdot}9 \pm 7{\cdot}1^{b}$
Blood FSH (ng/ml)	24	255 ± 59^{a}	90 ± 31^{b}	80 ± 48^{b}	35 ± 2^{b}
	48	170 ± 46^{a}	73 ± 10^{b}	98 ± 27^{ab}	$<35^{b}$
No. of oocytes shed per ovary		Not done	$3{\cdot}4 \pm 0{\cdot}4^{a}$	$4{\cdot}8 \pm 0{\cdot}3^{a}$	$16{\cdot}4 \pm 1{\cdot}7^{b}$

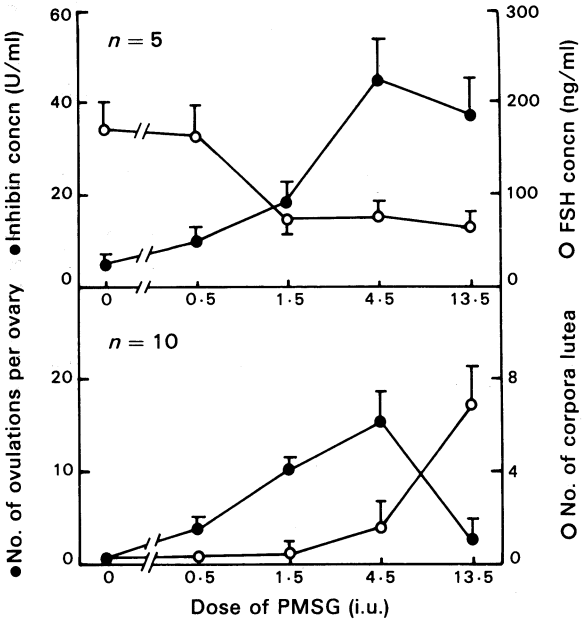

Fig. 1. Experiment 2: concentrations of peripheral blood inhibin, FSH, and ovulation rate of immature mice at 48 h after varying doses of PMSG injection. Values for inhibin (●) and FSH (○) are mean concentrations of blood samples pooled from three mice each (n = 5 pools in each case). Values for numbers of ovulations (●) and numbers of corpora lutea with entrapped oocytes (○) are means for individual mice (n = 10). Vertical bars indicate standard errors.

with 4·5 i.u. of PMSG. The concentrations of 36·8 ± 8·3 U/ml in mice given the highest dose of PMSG (13·5 i.u.) were not significantly different from those for the 4·5 i.u. dose. Peripheral FSH concentrations were 169 ± 25 ng/ml in control mice and were significantly reduced in mice which received 1·5, 4·5, or 13·5 i.u. of PMSG ($F_{4,16} = 9·31$, $P < 0·001$); FSH levels were 73 ± 13, 75 ± 8, and 64 ± 4 ng/ml respectively. The circulatory levels of inhibin and FSH were negatively correlated ($r = -0·534$, $n = 25$, $P < 0·01$).

There was a progressive increase in numbers of ovulations ($F_{3,12} = 37·3$, $P < 0·001$) over the first three doses of PMSG (0·5, 1·5 and 4·5 i.u.) but there was a sharp fall with the highest dose and the ovaries of this group of mice contained predominantly corpora lutea with entrapped oocytes (Fig. 1). Peripheral inhibin levels were positively correlated with the number of ovulations per ovary ($r = 0·543$, $n = 25$, $P < 0·01$) and with the number of corpora lutea with entrapped oocytes ($r = 0·448$, $n = 25$, $P < 0·05$).

Discussion

In this study we have induced ovarian follicular growth in immature mice by treatment with varying doses of PMSG. As a consequence of induced follicular growth, ovarian and circulatory levels of inhibin have been demonstrated to rise progressively with increasing doses of PMSG, similar to observations previously reported for immature female rats (Lee *et al.* 1981, 1982). Furthermore, peripheral FSH and inhibin concentrations were inversely related, in accord with the proposed feedback action of inhibin on pituitary FSH secretion.

A distinct finding in this study is the close relationship between ovulation rate and the concentration of inhibin in peripheral blood. Our original supposition, based on the observation (Lee *et al.* 1981, 1982) that only rats with ovaries stimulated to grow by PMSG exhibited raised circulating levels of inhibin, was that circulatory inhibin is derived predominantly from preovulatory growing ovarian follicles. The present study on immature mice confirms the studies on rats, and the close relationship between secreted inhibin and ovulation rate indicates more strongly the likely origin of inhibin from growing preovulatory follicles. Additional evidence to support this comes from studies in adult cycling rats and rats of various stages of sexual development. In normal adult cycling rats, ovarian and peripheral blood inhibin levels are highest during the early afternoon of pro-estrus (Lee *et al.* 1983), i.e. at a time when rapid growth of preovulatory ovarian follicles takes place. Additional evidence to indicate that growing preovulatory follicles are involved in the secretion of inhibin has been provided from experiments involving PMSG-primed rats which received a luteinizing dose of hCG (Lee 1983; Carson and Lee 1983). Rats treated with PMSG and which received hCG 40 h later showed abrupt falls in ovarian and circulating levels of inhibin, suggesting that luteinization of preovulatory follicles switched off inhibin biosynthesis and secretion (Lee 1983). Also, the treatment of PMSG-primed rats with hCG 4 h prior to isolation of ovarian follicles results in reduced follicular inhibin content and production *in vitro* (Carson and Lee 1983).

During pubertal development in female rats, ovarian and peripheral blood levels of inhibin increased progressively from the second post-natal week to reach maximum levels by 5 weeks after birth (Lee *et al.* 1984). The formation of an antrum in ovarian follicles of rats occurs from about 2 weeks of age (de Reviers and Mauleon 1979). By contrast, peripheral blood FSH and oestradiol concentrations decrease during the same

period (Dohler and Wuttke 1975), indicating that the rising circulating levels of inhibin (and not oestradiol) may well be responsible for the decreased FSH secretion during sexual maturation. Other investigators (Hermans *et al.* 1980) have shown an increase with age in pituitary sensitivity to an inhibin-like ovarian follicular fluid preparation in female rats. Taken together, the decreasing levels of FSH with age may well be due to increased pituitary sensitivity to inhibin and to rising circulating levels of inhibin. The granulosa cells of the ovarian follicle have been shown to be the source of ovarian inhibin (Erickson and Hsueh 1978; Lee 1984). It is likely that the increased secretion of inhibin during sexual maturation is due to increased numbers of granulosa cells within the growing ovary. Definitive studies to examined this point would require experiments to determine the secretion rate of inhibin from granulosa cells at various stages of sexual development.

In summary, the results of this study are consistent with the view that growing preovulatory follicles are responsible for the secretion of inhibin into the systemic circulation and the determination of inhibin levels in blood is possibly a useful index of preovulatory growth and ovulation.

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