Prevention of Aminoglutethimide Phosphate (AGP) Block of Ovulation in PMS-Treated Immature Rats[1] (35394)

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Aminoglutethimide phosphate (AGP) is an inhibitor of 20α-hydroxysteroid dehydrogenase necessary for mitochondrial conversion of cholesterol to pregnenolone (1–5). Administration of AGP markedly decreased ovarian tissue levels of progesterone and 20 α-dihydroprogesterone in immature rats superovulated with pregnant mare serum (PMS) and/or luteinizing hormone (LH (6). The mechanism of ovulation by PMS alone has been reported by many workers as due to the release of endogenous ovulating hormone (7–10). Ying and Meyer (11) have observed that the incidence of ovulation in 22-day-old rats, treated with various doses of PMS, reached a maximum at 3 and 30 IU. The effects of progesterone or other steroids on neurological control of ovulating hormone release and ovulation in 3-IU PMS-treated immature rats approximated the effects which were previously reported for the adult rat (11–13). The advancement and postponement of ovulation by progesterone injected at different times has been reported in both adult (14) and immature rats pretreated with PMS (13, 15). Furthermore, it has been suggested that the release of endogenous gonadotropin is regulated by the absolute amounts and/or ratio of sterioids (12). The current investigation was undertaken to examine the effect of AGP on PMS-induced ovulation in the immature rat and to determine whether steroids play a role on the hypothalamic activation of ovulating hormone release.

Materials and Methods. Female rats, 21 days old (body wt 50–55 g) obtained from the Holtzman Company, Madison, Wisconsin were caged in a light-controlled (14-hr light-10-hr dark), air-conditioned (73–78°F) room. Purina rat chow and tap water were provided *ad libitum.* All animals were injected subcutaneously with 3 IU of PMS (Equinex, Ayerst Laboratories, New York) in 0.25 ml of 0.9% NaCl between 7:00 and 8:00 a.m. on day 22. It has been shown that this treatment induces ovulation in 80% of the animals with an average of 5 ova/ovulating rat (11). To determine the effect on ovulation, a single injection of 1.0 mg of AGP (Ciba Pharmaceutical Company, Summit, New Jersey) was given subcutaneously in 0.25 ml of 0.9% NaCl at 9:00 a.m. on day 22 to 32 rats pretreated with 3 IU of PMS (Table I). Half of these animals were killed on day 25 (Group B) and the rest on day 26 (Group C).

In the second experiment, animals were divided into 6 groups (Table II). Two groups served as PMS and AGP controls. In Groups C and E, estradiol-17β (1 μg) dissolved in 0.1 ml of sesame oil or testosterone (0.5 mg) suspended in 0.25 ml of propylene glycol was given subcutaneously at 10:00 a.m. on day 22, respectively, to 3-IU PMS-primed rats without AGP treatment. Same amount of steroid was given at the same time to rats pretreated for the AGP block of ovulation (Groups D and F). All animals were killed on day 25.

In the third experiment 1.0 mg of AGP was given as in Expt. 1 to 27 animals pretreated with PMS. Nine of these animals were injected subcutaneously with 0.5 mg of progesterone in 0.1 ml of sesame oil at 10:00 a.m. on day 24. Eight received intravenous injections of 10 μg of luteinizing hormone (NIH-LH-S6) in 0.1 ml of physiological

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TABLE I. Effect of Aminoglutethimide Phosphate (AGP) on Ovulation in Immature Rats Pretreated with 3 IU of PMS.

Group	Treatment[a]	Day of autopsy	No. rats ovulated (%)	Av ovarian wt (mg ± SE)	Ova (av no./ovulating rat ± SE)
A	PMS	25	13/15 (87)	30.09 ± 1.49	5.23 ± 0.41
B	PMS + AGP	25	0/16 (0)	22.93 ± 0.74[b]	—
C		26	14/16 (88)	29.58 ± 2.08	5.57 ± 0.52

[a] Three IU of PMS or 1.0 mg of AGP dissolved in 0.25 ml of saline solution was given subcutaneously between 7:00 and 8:00 a.m., or at 9:00 a.m., respectively, on day 22.

[b] Significantly different from PMS controls ($p < 0.01$).

saline at 1:30 p.m. on day 24. Ten animals served as AGP controls. All animals were killed on day 25. On the day of autopsy, all animals were killed with an overdose of ether. The ovaries were removed and weighed to the nearest 0.1 mg on a Mettler analytical balance. Ovulation was examined by removing the oviducts and counting the ova by means of a light microscope as described previously (12).

Results. One mg of AGP, administered subcutaneously at 9:00 a.m. on day 22, inhibited ovulation in all of 16 rats treated with 3 IU of PMS on day 22 and killed on day 25 (Table I). However, ovulation occurred in 14 out of 16 rats killed on day 26. The percentage of rats ovulating and the average number of ova shed were not significantly different from those of PMS controls. The ovarian weights of animals treated with AGP and killed on day 25 were significantly lighter than those of PMS controls ($p<0.01$).

The inhibition of ovulation on day 25 by administration of AGP on day 22 could be prevented if steroid such as 1.0 μg of estradiol-17β, or 0.5 mg of testosterone was given subcutaneously at 9:00 a.m. on day 22 (Table II). Estradiol-17β, or testosterone given subcutaneously at 9:00 a.m. on day 22 to 3-IU PMS-primed rats without AGP treatment resulted in ovulation in over 70% of animals, indicating no significant difference from those of PMS controls. The same steroids administered after AGP injection in rats pretreated with 3 IU of PMS showed significant increase in the percentage ovulating over the PMS, AGP-injected controls. One μg of estradiol restored the ovulating ability of AGP-treated animals from 0 to 70% with an average of 4.6 ova. Testosterone (0.5 mg) also induced ovulation in 50% of the animals pretreated with PMS and AGP.

To determine if the ovaries of 3-IU PMS rats treated with AGP are mature enough to be ovulated, 0.5 mg of progesterone in 0.1 ml of sesame oil or 10 μg of LH in 0.1 ml of physiological saline was injected subcutaneously at 10:00 a.m. or intravenously at

TABLE II. Effect of Estradiol or Testosterone on the Postponement of Ovulation by AGP in Immature Rats Treated with 3 IU of PMS on Day 22.

Groups	Treatment[a]	No. rats ovulated (%)	Av ovarian wt (mg ± SE)	Ova (av no./ovulating rat ± SE)
A	PMS	8/10 (80)	29.55 ± 1.63	5.11 ± 0.48
B	+ AGP[b]	0/10 (0)	22.87 ± 1.05	—
C	+ estradiol,[c] 1 μg	7/9 (78)	35.46 ± 1.97	6.57 ± 0.37
D	+ AGP + estradiol, 1 μg	7/10 (70)	33.53 ± 2.55	4.57 ± 0.48
E	+ testosterone,[c] 0.5 mg	7/10 (70)	27.58 ± 0.98	5.57 ± 0.43
F	+ AGP + testosterone, 0.5 mg	6/12 (50)	26.58 ± 1.34	4.50 ± 0.34

[a] All animals were killed on day 25.

[b] One mg of aminoglutethimide phosphate was given at 9:00 a.m. on day 22.

[c] Estradiol-17β dissolved in 0.1 ml of sesame oil, or testosterone suspended in 0.25 ml of propylene glycol was given subcutaneously at 10 a.m. on day 22.

TABLE III. Induction of Ovulation by Progesterone or Luteinizing Hormone Injected on Day 24 in Immature Rats Treated with 3 IU of PMS and 1.0 mg of AGP at 22 Days of Age and Killed on Day 25.

Treatment	No. of rats ovulated (%)	Av ovarian wt (mg ± SE)	Ova (av no./ovulating rat ± SE)
PMS	8/10 (80)	29.55 ± 1.63	5.11 ± 0.48
+ AGP	0/10 (0)	22.87 ± 1.05[a]	—
+ progesterone[b]	9/9 (100)	32.42 ± 1.3	5.33 ± 0.41
+ LH[c]	8/8 (100)	29.57 ± 2.91	5.13 ± 0.64

[a] Significantly different from PMS controls ($p < 0.01$).

[b] 0.5 mg of progesterone in 0.1 ml of sesame oil was injected subcutaneously at 10:00 a.m. on day 24.

[c] 10 μg of NIH-LH-6 in 0.1 ml of physiological saline was injected intravenously at 1:30 p.m. on day 24.

1:30 p.m. on day 24, respectively. It has been shown that this amount of LH or progesterone induces ovulation or facilitation of ovulation in immature rats treated with a nonovulatory dose of PMS. Table III shows that both progesterone and LH caused 100% ovulation with an average of 5.3 or 5.1 ova, respectively, indicating that the follicles have been stimulated and are ready to respond to ovulating hormone.

Discussion. Previously, we have observed that injection of estrogen either stimulated or facilitated ovulation in immature rats receiving a subovulatory dose of PMS (unpublished data). However, a high dose of estrogen injected on the day of PMS administration in immature rats treated with 3 IU of PMS produced inhibition of ovulation. Furthermore, we have been able to induce ovulation in over 80% of immature rats 72 hr after the administration of a low, physiological dose of estrogen. Prevention of the AGP block of ovulation by estradiol as reported here confirms our previous observation and suggests that estrogen plays a physiological role in causing endogenous gonadotropin release.

It was not surprising that testosterone has the same effect as estradiol in preventing AGP block of ovulation, because proper administration of androgens has been shown to cause vaginal opening, onset of puberty, and ovulation in immature rats (16–23).

Increase of pituitary FSH release in immature female rats has been demonstrated with a single injection of androgen (24) or estrogen (25), as measured by ovarian weight changes following HCG augmentation. Moreover, Naqvi and Johnson (26) reported that a single injection of progesterone did not stimulate FSH output, and when given 1 hr before the estrogen, completely abolished its effect on FSH.

The action of 3 IU of PMS in the induction of ovulation in immature rats has been postulated as triggering the release of endogenous gonadotropins and subsequently ovulation (7–11). It has also been proposed that an optimum steroid environment is essential for the excitability of the hypothalamus which controls the release of gonadotropins (12).

It has been demonstrated that AGP has the ability to decrease side-chain cleavage of cholesterol in the rat ovary. However, a very rapid turnover of ovarian cholesterol in the absence of this compound was also noted (6). This may be interpreted as rapid synthesis of progestins in the absence of AGP. Recent reports of Wilks *et al.* (27) showed that pretreatment of rats with AGP resulted in a twofold enhancement of the stimulatory action of LH on steroidogenesis *in vitro*, mainly progestin. In immature rats primed with 30 IU of PMS, Zarrow and Hurlbut (28) suppressed ovulation with progesterone at the time of the PMS injection. Ying and Meyer (13) also demonstrated that treatment with progesterone 2 hr after the PMS injection postponed ovulation for 24 hr. Zarrow and Clark (29) have reported that aminoglutethimide prevented uterine growth in

gonadotropin-treated rats, presumably by inhibiting estrogen synthesis. It is postulated that the postponement of ovulation by AGP and the prevention of AGP block of ovulation by estrogen or androgen is due to the restoration of an optimum steroid environment, and consequent release of pituitary gonadotropin(s) resulting in ovulation.

Summary. Injection of 1.0 mg of AGP postponed ovulation for 24 hr in immature rats, pretreated with 3 IU of PMS, presumably due to inhibition of ovarian steroidogenesis. This block was completely prevented by estradiol and testosterone, administered 1 hr after AGP. Progesterone or LH administered on day 24 also prevented the block of ovulation by AGP.

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