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Photoperiod Effect on Gonadotropin Releasing Hormone Induced Ovulation in the Immature Rat[3]

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ABSTRACT

Immature female rats exposed to constant light from birth, ovulated by 35 days of age in response to 1000 ng of gonadotropin-releasing hormone (GnRH). Animals reared in a 14 h light:10 h dark photoperiod did not ovulate by 37 days of age unless previously primed with PMS. Constant light or PMS administration decreased the age at which large ovulatory follicles first appeared, therefore, possibly explaining the ability of GnRH to induce ovulation at an earlier age in these animals. Constant light, however, inhibited the ovulatory response to PMS alone. GnRH can override this inhibition indicating that constant light alters the ability of the hypothalamus and/or other CNS components to regulate GnRH secretion.

INTRODUCTION

Among the exteroceptive influences which stimulate the initiation of gonadal function, photoperiod appears to be among the most important (Critchlow, 1963). Prepubertal female rats, exposed to constant light, experience vaginal opening earlier than do those exposed to a light/dark schedule; while sexual development is retarded in rats reared in darkness (Fiske, 1932, 1939, 1941; Luce-Clausen and Brown, 1939) or blinded by optic enucleation (Browman, 1940; Truscott, 1944). Exposure to constant illumination also has been shown to alter pituitary gonadotropin levels (Fiske, 1941).

Ovulation can be induced in immature rats as early as 25 days of age with either a single injection of pregnant mare's serum gonadotropin (PMS) (McCormack and Meyer, 1962) or as early as Day 23 with a single injection of human chorionic gonadotropin (HCG) (Sugawara and Takeuchi, 1970). Administration of PMS and HCG in combination brings about ovulation in 13–14 day old rats (Zarrow and Wilson, 1961). Induction of precocious ovulation with PMS in the immature rat is biphasic; the first ovulation occurring within 24–36 h after PMS injection and the second ovulation by 72 h of injection (De La Lastra et al., 1972). The initial ovulation is independent of, but probably assisted by pituitary luteinizing hormone (LH) release; whereas the second ovulation requires an intact pituitary. The second ovulation in response to PMS can be prevented by hypophysectomy or pharmacological blocking agents administered immediately before the expected ovulatory surge of LH (Zarrow and Quinn, 1962). Constant illumination also renders immature rats unresponsive to PMS (Wagner and Brown-Grant, 1965). Ovulatory failure in such animals cannot be attributed to lack of ovarian responsiveness since HCG administration results in ovulation.

The administration of gonadotropin releasing hormone (GnRH) induces a LH surge and ovulation in adult rats (Martin et al., 1974), and LH release in male and female rats of various ages (Debeljuk et al., 1972; Spona and Luger, 1973). The effect of constant illumination on the onset of puberty, suggests the possibility that the competence of prepubertal rats to ovulate in response to GnRH might be modified by the photoperiod.

The objectives of this study are threefold: 1) to examine the effect of constant illumination on the age at which GnRH induced-ovulation first occurs, 2) to determine whether or not

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such ovulation is advanced or enhanced by PMS, and 3) to determine if the light induced blockade of PMS evoked ovulation can be overcome by GnRH administration.

MATERIALS AND METHODS

Adult female Wistar rats were caged in a controlled environment (photoperiod 14 h light/10 h darkness, lights on at 0600, temperature 24°C, humidity 45 percent). Those females with regular estrous cycles were caged with a male and vaginal smears continued daily until spermatozoa were detected in the vaginal lavage (designated Day 1 of pregnancy). Five days prior to the anticipated date of delivery, pregnant females were placed in large transparent plastic cages containing nesting material. Beginning on Day 21 of gestation, rats were inspected periodically between 0800 and 1800 for arrival of litters. Those litters found at 0800 were considered to be born that day. Rats were randomly assigned to either the control light schedule (L/D:14/10) or transferred to the experimental light regime of constant illumination (L/L). The respective animal rooms were essentially identical save for the photoperiods. Within 4 days of birth, litters were reduced to 8 pups; weaning was at 22 days of age. Beginning at Day 35 of age, nine untreated litters, which were also reduced to 8 pups by Day 4, were examined daily for the occurrence of vaginal opening (LD 5 litters, LL 4 litters).

Following placement in either the control or experimental photoperiod, individual rats within each litter were assigned to one of four treatment groups: 1) PMS-treated, 2) GnRH-treated, 3) PMS + GnRH-treated, and 4) saline-treated (control). Rats in the PMS or PMS + GnRH treatment groups received 5 IU of Equinex (Ayerst) intraperitoneally between 1300–1400 h. Saline controls and GnRH treated rats received 0.1 ml of physiological saline. Forty-eight hours later, rats were weighed and anesthetized (25 mg/kg body weight pentobarbital supplemented with ether); the external jugular vein was exposed and 0.4 ml of blood was collected. Immediately following the blood collection, rats received either 1000 ng of synthetic gonadotropin releasing hormone (GnRH, Ayerst Ay-24, 031) in 0.1 ml of saline or an equivalent volume of saline alone via the exposed vein. Twenty minutes after injection, a second blood sample (0.4 ml) was collected. Serum was prepared from each blood sample and stored at −20°C for LH radioimmunoassay. LH levels in these rats will be the subject of a subsequent communication. Following recovery from anesthesia, rats were returned to their respective photoperiod environments. Eighteen hours after GnRH or vehicle administration, animals were examined for the presence of vaginal opening, then anesthetized with ether and decapitated.

The uterus was removed, dissected free of adhering tissue, blotted to express fluid and weighed. The ovaries and attached oviducts were removed and trimmed of fat. Oocytes were flushed from the oviducts with saline. Only those animals in which the oocytes were found in the cumulus mass were considered to have ovulated within the previous 18 h. Following flushing, the oviducts were removed and ovarian weight recorded. Ovaries were fixed in Bouin's solution, embedded in paraffin, sectioned at 15u, stained with hematoxylin and eosin, and subjected to histological examination.

Responses of control and experimental groups were compared statistically by Student's *t* test. The percentage of rats ovulating in each group as well as vaginal opening was analyzed by chi-square. A p value of less than 0.05 was considered significant.

RESULTS

Vaginal Opening

In most nontreated LL rats (23/27) vaginal opening occurred on Day 38 (Range 37–40); while nontreated LD rats (25/33) exhibited vaginal opening on Day 42 (Range 39–44). This difference was highly significant ($P<0.01$). PMS significantly increased the incidence of vaginal opening by Day 37 in the LD animals and by Days 31, 35 and 37 in the LL animals (Figs. 1 and 2). GnRH increased the number of LL animals with vaginal opening by Day 37 while a combination of PMS and GnRH was

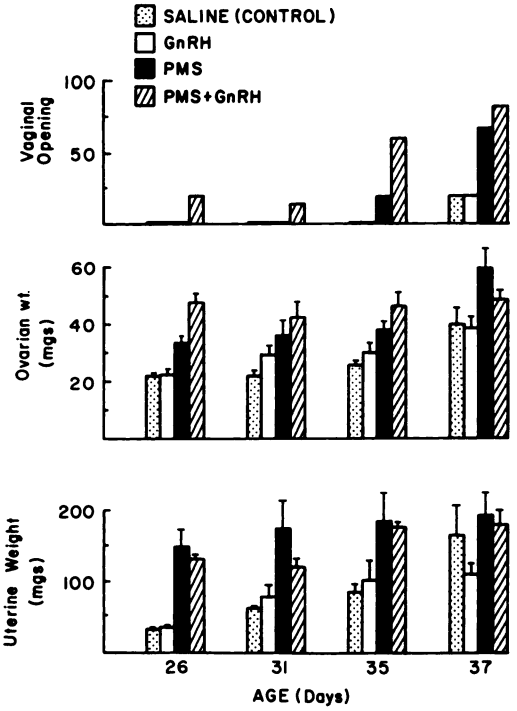

FIG. 1. Effects of GnRH, PMS, and PMS plus GnRH on vaginal opening, ovarian weight, and uterine weight in the immature rat housed under a light-dark (LD) regime. The number of rats with vaginal opening by the time of autopsy is expressed as percent while ovarian and uterine weight are expressed as the mean ± standard error. Values represent 5–8 animals per group.

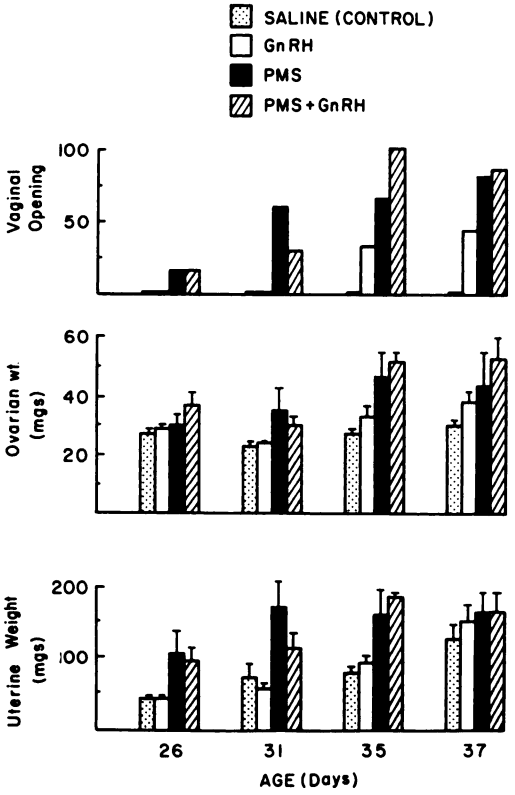

FIG. 2. Effects of constant light (LL) on the immature rat. For details see Fig. 1.

effective by Day 35 and 37 in both LL and LD rats. When compared to nontreated control, saline injections did not advance vaginal opening.

Ovulation

GnRH alone induced ovulation in LL rats when given on Day 35 or 37 but not at any day tested in LD rats (Table 1). Ovulation was induced by PMS from Day 31 to 37 in LD rats and on Day 35 and 37 in LL rats. A combination of PMS and GnRH induced ovulation in rats 26 days of age. However, this treatment was significantly ($P<0.05$) more effective in the LD rats. Although the number of PMS and GnRH treated LD rats that ovulated varied with age, this difference did not prove to be significant. An increase in the percentage of ovulating rats was seen after Day 26 in the PMS and GnRH treated LL rats.

Ovarian Histology

Medium size (250 u) antral follicles were first noted in 35 day-old LD rats. With the exception of the large preovulatory follicles (>500u), all other types of follicles were present by Day 37 in most LD animals. These observations are consistent with previous reports (Meijs-Roelofs et al., 1973). The sequence of follicular growth and development in LL animals were similar to the LD animals but advanced by approximately 5 days.

PMS acted on 26 day LD rats to increase the number of large follicles, the amount of interstitial tissue, and the thickness of the theca. Some ovaries from 26 day-old PMS treated LD rats possessed follicles in which the oocytes had resumed meiosis, cumulus cells were dispersed and the granulosa layer thinned. Although these

TABLE 1. The effect of photoperiod on percent of ovulation after treatment with pregnant mare's serum (PMS) and/or gonadotropin releasing hormone (GnRH).

Treatment	Age at GnRH injection (days)			
	26	31	35	37
		Light/Dark		
Saline (Control)	0	0	0	0
GnRH	0	0	0	0
PMS*	0	17	40	50
PMS and GnRH*	100	57	100	67
		Light/Light		
Saline (Control)	0	0	0	0
GnRH	0	0	17	43
PMS*	0	0	17	40
PMS and GnRH*	17	43	100	67

*5 IU PMS given 48 hrs. prior to 1000 ng of saline or GnRH administration. Animals autopsied 18 h after saline or GnRH injection. 5-8 animals per group.

follicles showed what appeared to be preovulatory changes (Vermeiden and Zeilmaker, 1974) they had not ovulated at the time of autopsy. Similar morphological conditions were also noted in the 31, 35 and 37 day old PMS treated LD rats. In the ovaries of the 35 and 37 day-old rats receiving PMS, two distinct degrees of luteinization was observed. One type of corpora lutea were completely luteinized (Fig. 3) while another type had a luteinized outer layer surround a mass of granulosa cells (Fig. 4).

PMS administered to the LL animal had effects similar to those in the LD animals with two exceptions. First, in LL rats of 31, 35 and 37 days of age, partially luteinized follicles with entrapped atretic oocytes were present in more than 50 percent of those animals (Figs. 5, 6). Entrapped ova were found in only one LD rat. Second, the two distinct types of corpora lutea that were found in the 35 and 37 day-old PMS-LD rats were observed only at 37 days in the LL rats.

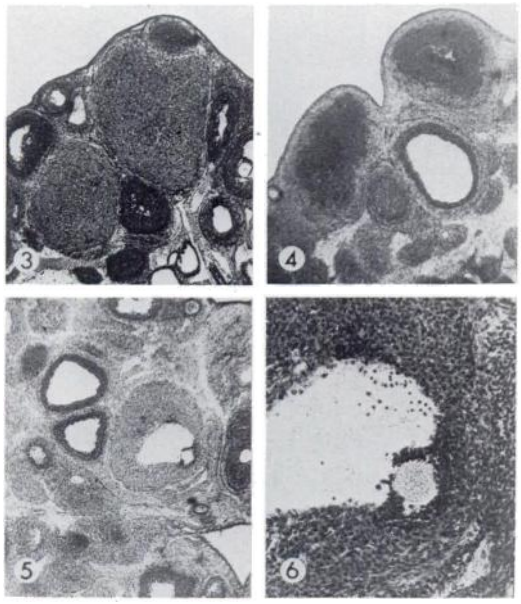

FIG. 3. Corpora lutea found in rats treated with PMS or PMS and GnRH are completely luteinized and probably result from the first wave of ovulation due to PMS. (75X)

FIG. 4. Corpora lutea associated with a GnRH induced ovulation. Note the presence of darker staining granulosa cells surrounded by luteal cells. (75X)

FIG. 5. An entrapped oocyte within a luteinized follicle. These follicles were found frequently in the ovaries of constant light animals treated with PMS and/or GnRH. (75X)

FIG. 6. Higher magnification of a entrapped oocyte. Although granulosa cells surround the oocyte, the majority of cells have luteinized. (300X)

GnRH alone induced ovulation in 35 and 37 day-old LL rats. Partially luteinized follicles without entrapped ova as well as entrapped ova in luteinized follicles were observed in these animals. In PMS and GnRH treated LL rats, follicles with preovulatory changes were seen in those animals that did not ovulate from Day 26 onward.

Ovarian and Uterine Weight

Ovarian weight remained fairly constant between Days 26 and 35 in both the LL and LD rats (Figs. 1, 2). Between Day 35 and Day 37 there was a sudden increase in ovarian weight in LD rats, but not in LL rats. PMS increased ovarian weight over non-treated controls ($P<0.01$) at all ages in both LL and LD rats with the exception of 26 days in the LL group. GnRH administration did not cause significant changes in ovarian weight in either PMS-primed or nonprimed rats.

The pattern of uterine weight gain was not affected by photoperiod (Figs. 1, 2). Uterine weight was increased by PMS treatment on Days 26, 31 and 35 but not on Day 37 in both LD and LL rats. GnRH injection resulted in a decreased uterine weight in LD and LL primed animals at Day 31 and in non-primed LD animals at Day 37.

Body weight was not significantly different between any treatment group on a given day of age.

DISCUSSION

A single iv injection of GnRH (1000 ng) results in ovulation in 100 percent of both Nembutal-blocked proestrous or diestrous adult rats (Martin et al., 1974). This dose of GnRH was ineffective in inducing ovulation in the prepubertal rat unless the rat has been previously primed with PMS or exposed to constant illumination. In the immature rat, housed under a light/dark regime, GnRH had a negligible effect on ovarian weight, uterine weight, ovarian histology and time of vaginal opening. However, when GnRH is given three times daily for a three day period, uterine weight increased and vaginal opening was advanced in 34 day-old rats (Schroder et al., 1973). A single injection of GnRH in mature female rats releases a single pulse of gonadotropin (Libertun et al., 1973), and presumably has the same effect in the

immature rat. Thus, these differences may be due to the total dose and route of administration of GnRH.

GnRH can induce ovulation in the 26 day-old LD rats primed with PMS. This indicates that GnRH can release gonadotropins as early as Day 26 in the PMS-primed rat. By Day 26, large follicles were present in the PMS-treated rats. Pretreatment with FSH induces LH receptors in the granulosa cells of antral follicles (Zeleznik et al., 1974). Possibly, PMS induced LH receptors within the follicles of 26 day-old rats which enable them to ovulate in response to a GnRH-induced LH surge. Although ovulation can occur by 26 days of age in the PMS primed animals, failure to induce ovulation in the 26-day old nonprimed rats is probably due to the inability of the ovary to respond to LH. However, the possibility exists that PMS could also be stimulating the hypothalamic-pituitary axis to release gonadotropin in response to GnRH. These effects could be mediated by PMS or a PMS-enhancement of estrogen secretion.

PMS given on Day 30 causes an LH peak on the afternoon of Day 32 and ovulation that evening (Hillensjö et al., 1974). The inability of PMS to induce ovulation in 100 percent of our animals could be due to 1) a relative low dose (5 IU) of PMS was given, and 2) completely luteinized corpora lutea, which possibly resulted from an earlier PMS-induced ovulation were observed in these rats. Ova from this PMS-induced ovulation were not always collected. When collected, they were not enclosed by cumulus cells and were fragmented, indicating ovulation probably took place shortly after PMS administration. In a few animals, however, "old" corpora lutea as well as "new" incompletely luteinized corpora lutea were present in the same ovary indicating that two waves of ovulation may have occurred.

Exposure to constant light has long been known to cause constant estrus in the adult and to advance the time of vaginal opening in the prepubertal rat (Critchlow, 1963). In the adult rat, constant light causes an increased serum FSH and prolactin level (Kledzik and Meites, 1974). If constant illumination also increases serum FSH levels in the immature rat, then the increase in FSH levels might be comparable to the PMS pretreatment; thereby increasing the effectiveness of GnRH on the ovulatory process in the immature animal. The fact that the sequence of follicular maturation was moved forward by constant light supports this hypothesis. However, the effect of constant light on the pituitary release of LH in response to exogenous GnRH must also be considered.

Even though the constant light animals ovulated in response to GnRH alone, ovulation was abnormal, as indicated by the presence of entrapped ova which possibly resulted from either an insufficient LH surge, an altered LH/FSH ratio or an incomplete ovarian response. The inhibitory effects of constant light on the ovulatory process were observed at Day 31 and 35 in the animals given PMS. Since HCG can overcome this blockade, constant light may effect the hypothalamus and/or pituitary (Wagner and Brown-Grant, 1965). However, GnRH treatment can also override this inhibition of ovulation. This suggests that constant light possibly interferes with the GnRH regulatory mechanism of the hypothalamus and/or other CNS components. Whether this interference is due to a direct neural effect or to indirect factors; such as estrogen production must be further investigated.

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RECOMMENDED REVIEWS

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