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STUDIES ON THE MECHANISM OF THE ONSET OF PUBERTY IN THE FEMALE RAT*

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SUMMARY

The effect of 5α-androstane-3α,17β-diol (3α-androstanediol) and its 3β epimer on the mechanism of the onset of puberty has been investigated. In the course of the study it was found that in two strains of rats examined, in the majority of animals vaginal opening is not accompanied by ovulation, nor is the presence of corpora lutea an indication for the first ovulation, whereas significant uterine weight increase is a correlate for the first presence of tubal ova. The first preovulatory LH surge was also found to occur on the day preceding the first cornified smear which was not the day of vaginal opening.

A daily dose of 25 μg of 3β-androstanediol from the 21st day up to vaginal opening advanced the first ovulation. 3α-androstanediol was found to be effective in inhibiting postcastrational LH release in immature female rats, while the 3β epimer was inactive in this respect. The 3β epimer advanced vaginal opening by an effect mediated by the ovaries. The origin of the secretion of these steroids was found to be ovarian, since ovariectomy reduced their concentration in peripheral circulation to an undetectable level. The possibility that these compounds participate in the mechanism of the onset of puberty is discussed.

In the female rat puberty may be defined as the developmental stage at which cyclic ovulation is initiated. In accordance with available information it appears that the gonads, reproductive tract, hypophysis and hypothalamus are normally capable of adult activities prior to puberty, and the initiation of function in these structures awaits only activation by appropriate hormonal stimuli. The prevailing explanation for onset of puberty is based on the observation that the pituitary-gonadal system in immature rat is more sensitive to the inhibitory effects of gonadal stimulation than that of the adult (see reviews 1, 2). Experiments in which blood LH levels were measured by radioimmunoassay (RIA) confirm this inhibitory hypersensitivity of the LH release mechanism [3]. This consideration led several authors to suggest [4–6] that the onset of puberty might be determined by an upward shift in threshold of the steroid-gonadotropin negative feedback mechanism, leading to increased gonadotropin secretion which precipitates puberty.

This explanation for the mechanism of the onset of puberty has to be modified, since the assumption that gonadotropin secretion is increased at the time of puberty had to be abandoned: determination of LH and FSH by RIA showed that these hormones are present in the circulation of immature rats in even higher concentrations than in the adult, and that their concentrations change only slightly at puberty [7, 8]. Furthermore, Swerdloff *et al.*[9] could find no difference in the threshold for the negative feedback of estrogen on gonadotropin release when comparing immature and mature rats. De Hertogh *et al.*[10] found that the metabolic clearance rate of estradiol-17β and estrone in immature rats is much smaller than that in adult animals, a finding that may explain the apparent hypersensitivity of the negative feedback in immature rats.

In search of an explanation for the mechanism of the onset of puberty, it was shown in our laboratory that systemic administration of 5α-androstane-3β, 17β-diol (3β-androstanediol) advances vaginal opening in rats, while its 3α epimer is inactive in this respect [11]. It has also been demonstrated that these two steroids are present in high concentrations in peripheral blood of immature rats, and disappear after onset of puberty [12]. From these observations it seems likely that they are involved in the normal onset of puberty.

Vaginal opening, first estrus and first ovulation

Time of sexual maturation is usually estimated by parameters such as time of vaginal opening, the age at which an estrous smear is first observed, or the presence of corpora lutea. In the majority of rats, vaginal opening, first estrus and first ovulation occur in that sequence within a 24-h period [2]. We thought it of interest to confirm this relationship by observing the first appearance of tubal ova. This was done in a Wistar-derived strain and in Charles River® rats. The stages of changing vaginal cytology observed in the different groups were variable, but vaginal opening in the majority of rats was neither accompanied by a fully cornified smear, nor by ovulation. Only when vaginal opening occurred after the 40th day of age (in these specific groups of rats), it was usually

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Table 1. Effect of 5α-androstane-3β,17β-diol on vaginal opening and on ovulation. 8 rats per group were injected daily with the dose dissolved in 0·2 ml sesame oil, up to vaginal opening. Results are expressed as mean ± standard error. Underlining means, significantly different from oil-injected controls ($P < 0·02$)

Treatment	Day of vaginal opening	Day of ovulation	No. of ovulated eggs
Oil-injected control	36·3 ± 0·6	43·8 ± 0·3	8·5 ± 0·5
25 μg	35·3 ± 0·9	40·3 ± 0·2	9·6 ± 0·3
100 μg	30·3 ± 0·4	42·8 ± 0·3	10·4 ± 0·3
400 μg	26·6 ± 0·1	47·8 ± 0·5	4·7 ± 1·1

accompanied by estrus and ovulation. In the majority of rats, at vaginal opening, a non-typical smear was found in which cornified cells and leucocytes together with granulated epithelial cells were present in different proportions. This smear endured from 2 to 5 days or longer. The oviducts at laparatomy were free of eggs till the first fully cornified smear. A similar description of events taking place in mice preceding the first ovulation was recently given by Stiff *et al.*[13]. As shown in Table 1, daily treatment with 25 μg of 3β-androstanediol from day 21 up to vaginal opening significantly advanced ($P < 0·01$) the first ovulation, whereas a daily dose of 400 μg induced precocious vaginal opening ($P < 0·01$), but delayed the first ovulation ($P < 0·01$) and reduced the number of eggs ovulated ($P < 0·01$), probably by inhibiting gonadotropin secretion.

In a group of rats the relationship between organ weight, presence of corpora lutea and ovulation was investigated. In 2 out of 4 rats killed before vaginal opening, well developed corpora lutea were found, while eggs were not detected in their oviducts. In rats with fully cornified smears ova were constantly present in the oviducts. In 3 out of 5 rats killed at different intervals before their first fully cornified smears, corpora lutea were present, without eggs being found in their oviducts. Data collected at necropsy are presented in Table 2. It can be seen that the only difference between rats before and after ovulation is the large increase in uterine weight in those rats in which ovulation was recorded. Thus, the presence of corpora lutea in peripubertal rats seems not to be a reliable indication for the first ovulation, whereas significant uterine weight increase is a correlate for presence of tubal ova.

Time course of serum LH levels during onset of puberty

The ovulatory surge of LH in the cycling rat occurs during a restricted period of 2 to 5 PM of the day of proestrus. In the immature rat, too, the first ovulatory LH surge occurs at the critical period, since pentobarbital blockade at 2 PM of the second day following PMS treatment of immature rats, prevents the first ovulation [14]. Pentobarbital treatment only at 2 PM on the second day after estradiol benzoate administration to immature rats prevents ovulation [15]. In order to confirm the dissociation found between vaginal opening and the time of ovulation, we determined the day of the first LH surge (Eckstein, Koch and Karni, unpublished results). Serum LH levels at the age of 21 to 50 days were determined by RIA in two separate experiments. In the initial experiment, rats were bled by cardiac puncture at 5 p.m. every 3rd day. This schedule of sampling was shown not to interfere with normal weight gain and with the time of vaginal opening. In the second experimental group the rats were bled only once in two weeks. In both groups, vaginal opening was not preceded by an increased level of serum LH. The first preovulatory LH surge was in all rats examined at the day preceding a fully cornified smear (Fig. 1).

Effect of the androstanediols in suppressing postcastrational LH release

Numerous studies since the early thirties have indicated the existence of a functional interrelationship between gonads and hypophysis in immature rats [1]. Recently, Caligaris *et al.*[16] showed that the gonadal-hypophysial mechanism for the tonic release of LH is already functioning in the 10-day-old rat, while

Table 2. Organ weight of rats at the peripubertal period in relation to ovulation. The numbers are mean ± standard error. Underlining means, significantly different from the group "Before ovulation". ($P < 0·01$)

Group	N	Day of necropsy	Body weight (g)	No. with corpora lutea	Uterus (mg)	Ovaries (mg)
Before ovulation	9	41·2 ± 2·1	97·4 ± 5·8	5/9	79·0 ± 13·5	27·1 ± 2·0
Opened at estrus with tubal ova	9	42·2 ± 2·3	115·7 ± 5·1	8/9	142·2 ± 6·8	22·8 ± 3·5
After ovulation	10	43·4 ± 3·4	112·9 ± 4·0	10/10	168·1 ± 17·4	31·7 ± 5·1

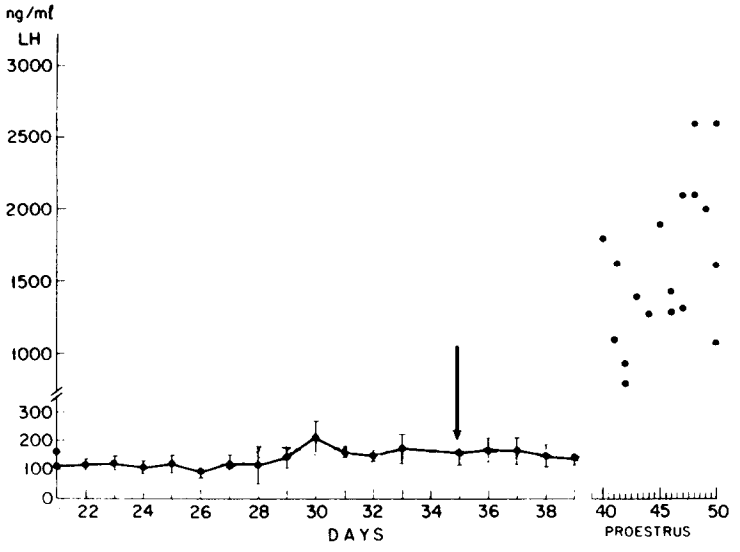

Fig. 1. Time course of serum LH concentration in normal rats. All the values are presented until the 40th day; thereafter, with the appearance of the first LH surge, only proestrus values are shown (as dots). Each point represents the mean ± standard deviation of 4 to 7 rats. Mean day of vaginal opening (34·9 ± 1·6) is indicated by an arrow.

this mechanism for the phasic release of LH starts to be functional only at the age of 22 25 days.

Estrogen and progesterone cannot be the only gonadal steroids involved in regulating LH release, as these gonadal steroids do not reproduce the fall in pituitary LH and the rise in plasma LH seen in the estrogen-treated castrated rat [17, 18, 5]. Therefore, it has been assumed that there is a synergism between estrogen (and progesterone) and some other, as yet unidentified, ovarian steroid(s). Thus, while the existence of a feedback relationship between ovaries and hypophysis in the immature rat has been established, the ovarian steroid(s) participating in inhibition of gonadotropin secretion in the prepubertal animal has not been identified. Therefore, we thought it of interest to see whether the androstanediols that are present at high concentrations in blood of immature rats can prevent postcastrational LH elevation.

Rats were ovariectomized at the age of 20 days and injected daily from the 21st to the 50th day of age either with 3α or 3β-androstanediol or estradiol benzoate [19]. Blood was drawn (at 8 to 10 AM) every 5th day throughout the experimental period for LH determination by RIA. As seen in Fig. 2 (A and B), plasma LH levels at 5 days following castration were already significantly elevated. All the 3 concentrations of 3β-androstanediol tested were without any effect in depressing the postcastrational LH release, while the 3 concentrations of 3α-androstanediol were effective in this respect.

At the end of the experiment, organ weights were recorded. Table 3 shows that 3β-androstanediol is devoid of any uterotrophic activity at the doses examined, while 3α-androstanediol is uterotrophic at the dose of 100 μg per 100 g body weight. It can also be seen that the effect of 3β-androstanediol on inducing precocious vaginal opening is mediated by the ovary, since it had no effect in ovariectomized rats even at the highest dose examined. The 3α epimer, on the other hand, has a direct effect on vaginal opening.

Origin of the secretion of 3α and 3β-androstanediol

Both androstanediols have been found to be present in peripheral blood of immature rats and were undetectable after onset of puberty [12]. Since both steroids seem to be involved in the mechanism of the onset of puberty, it was of interest to determine their origin in the immature rats.

Ovariectomy at the age of 19 days resulted in the disappearance of both compounds from peripheral blood of 23 day old rats, while in intact rats and in sham-ovariectomized animals the level of 3α and of 3β-androstanediol were about 150 and 100 ng per ml, respectively [20]. These are high levels of steroids, when compared to either a level of less than 10 pg/ml of estradiol-17β at the age of 30 to 33 days [21], or to the level of 1 to 2 ng per ml testosterone in prepubertal rats determined by RIA [22].

In incubations of immature rat ovaries with labeled pregnenolone, the 3α-form was found to be the major metabolite, whereas 3β-androstanediol could not be detected [23]. This made the ovarian origin of 3β-androstanediol questionable, since we suspected that 3α-androstanediol could be produced by extraovarian epimerization of the 3α epimer, whose ovarian origin was established (23, 24 and Lerner and Eckstein, unpublished results). To resolve this question we injected 3α-androstanediol into ovariectomized rats and determined the quantity of 3β-androstanediol in peripheral blood. In two separate experiments, about 15% of the 3α-form was epimerized peripherally to the 3β

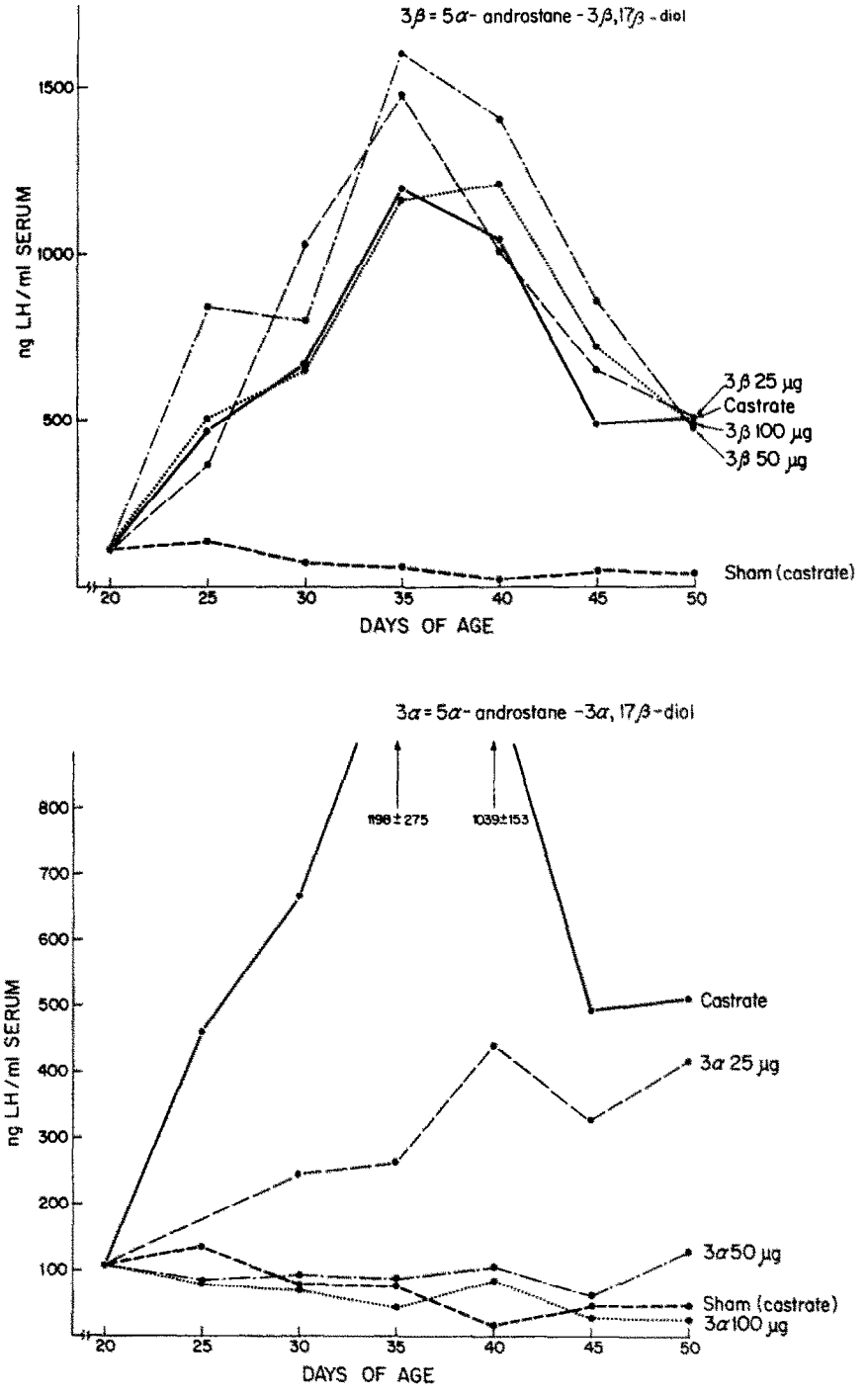

Fig. 2. (a and b). Time course of serum LH in rats ovariectomized or sham-operated on day 20 and injected daily with 3β-androstanediol (a), or 3α-androstanediol (b) from the 21st to 50th day of age. Steroids were injected in the specified doses per 100 g body weight.

epimer. Since the ratio of 3α/3β found in peripheral blood at 22 to 24 days is 0·9 to 1·1, a part of the 3β-androstanediol found in peripheral blood of immature rats is, maybe, of ovarian origin [20].

DISCUSSION

In our local Wistar-derived strain, as well as in Charles River® rats, normal vaginal opening is not necessarily correlated with the first ovulation. A lag period of up to 5 days (and more) between these events is observed in most animals. This statement is based on observing the first appearance of tubal ova and the occurrence of the first ovulatory LH-surge. The presence of corpora lutea in peripubertal rats is, in these strains, no reliable indicator for the first ovulation, whereas a significant uterine weight

Table 3. Effect of 3α and 3β-androstanediol and of estradiol benzoate on uterine weight and on day of vaginal opening in ovariectomized rats. Rats were castrated on day 20 and injected daily with the steroid dissolved in 0·1 ml of sesame oil, up to day 50, when uterine weight was recorded. Numbers are mean ± standard error

Treatment	N	Uterine weight (mg)	Day of vaginal opening	Vagina closed on day 50
Sham-castrated	6	252·5 ± 29·5	38·1 ± 2·8	0
Castrated	8	30·7 ± 3·6	43, 45	6
3α-androstanediol				
25 μg/100 g B.W.	9	31·1 ± 3·7	44, 4 × 48	4
50 μg/100 g B.W.	8	46·0 ± 6·4	43·3 ± 1·5	0
100 μg/100 g B.W.	8	48·2 ± 4·7	37·0 ± 2·1	0
3β-androstanediol				
25 μg/100 g B.W.	6	36·0 ± 5·9	43, 41, 2 × 44	2
50 μg/100 g B.W.	10	35·0 ± 4·9	39, 40, 2 × 42, 3 × 44	3
100 μg/100 g B.W.	9	32·8 ± 2·6	41, 47, 5 × 44	2
Estradiol benzoate				
50 ng/100 g B.W.	8	121·2 ± 9·8	34·2 ± 1·1	0
100 ng/100 g B.W.	6	146·3 ± 11·5	31·3 ± 0·7	0

Underlining means, significantly different from castrated group, ($P < 0·01$).

increase (elevated level of estrogen?) invariably proceeds the first ovulation.

Up till now estrogen and gonadotropins are the only compounds known to induce precocious ovulation in the immature rat. Estrogens were known to have this ability since the discovery of this phenomenon by Hohlweg [25]. Recently, Ying and Greep[15] have demonstrated that ovulation can be induced in a high percentage of immature rats after a single dose of estradiol benzoate. Induction of ovulation in immature rats by PMS treatment has been the subject of many studies since the initial finding by Cole[26]. By this treatment, the ovaries produce estrogen that leads to a preovulatory LH surge [27]. This sequence of events has been shown by experiments in which injections of antisera against estradiol-17β administered up to 15 h prior to the expected LH release, blocked PMS-induced ovulation [28].

It seems that the final stages in the sequence of events leading to sexual maturation occurring in rats (estrogen secretion for triggering the LH-surge), are the same as the stages preceding ovulation in the cycling rat [29]. The earlier events in the sequence which are unique for the first ovulation, remain to be elucidated. To the best of our understanding, the androstanediols participate in one or more of these stages, for the following reasons:

1. 3β-androstanediol advances the first ovulation. This and precocious vaginal opening are achieved even though the steroid is not uterotrophic, in other words, by stimulating a stage in the onset of puberty earlier than the stage stimulated by estrogen.

2. An inhibitory ovarian feedback mechanism for LH release is operative in the immature rat. Estrogen (and progesterone) cannot be the only ovarian steroids regulating LH release (as discussed earlier). On the other hand, 3α-androstanediol is effective in suppressing postcastrational LH release in immature female rats; is produced by the ovaries and is present in high concentrations in peripheral blood till puberty. Therefore, it seems logical to assume that it participates in regulating LH release before onset of puberty.

3. Vaginal opening by itself is no indication of puberty, but it is a prerequisite for it. Administration of 3β-androstanediol to immature rats advanced vaginal opening up to 10 days. This effect is mediated by the ovaries, since it is abolished after ovariectomy.

4. Both androstanediols are present in peripheral blood of immature rats in high concentrations and disappear after onset of puberty. They originate from the ovaries. The 3α-androstanediol is the major metabolite of pregnenolone in incubations of immature rat ovaries, and is undetectable with the same conditions of incubation when adult ovaries are used [24].

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