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# Naloxone Administration to Female Hamsters Advances Puberty by Enhancing Luteinizing Hormone Release (42341)

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*Abstract.* In the female hamster, a daily rhythm of gonadotropin release begins almost 3 weeks prior to the initiation of 4-day estrous cycles. A temporal relationship exists between the onset of this cyclic release of gonadotropin and age at puberty. We hypothesized that since opiate agonists depress circulating gonadotropins and antagonists increase them in both adult and immature rodents, endogenous opiates may influence the mechanism controlling cyclical gonadotropin release in the prepubertal femal hamster and thus affect rate of sexual maturation and hence the age at puberty. This proposal was tested by chronic administration of naloxone (NAL), an opiate receptor antagonist. We predicted that NAL might induce the early initiation of daily surges of luteinizing hormone (LH) if endogenous opiates inhibit sexual maturation. Naloxone was injected daily (50 mg/kg body wt) at about 1300 hr from Days 1 through 17 of age. The NAL injections increased serum LH and significantly advanced the age at which first estrus vaginal discharge was observed (32 vs 38 days for saline-injected controls in Experiment I and 31 vs 37 days in Experiment II). However, the NAL injections did not correspondingly advance the age of initiation of endogenously generated daily cycles of circulating LH. We conclude that blockade of opiate receptors accelerates sexual maturation by directly inducing the release of LH and not by advancing the age of initiation of endogenous gonadotropin surges. 

Cyclic release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) precedes puberty by 2 to 3 weeks in the female hamster (1). The daily rhythms of gonadotropins begin on the 16th or 17th day of life and are characterized by a peak occurring at 1700 hr and relatively low levels at other times of the day and night. The role of the daily gonadotropin surges in the regulation of sexual maturation is uncertain. However, their initiation is followed in a few days by a large increase in ovarian progesterone secretion, which rapidly becomes cyclical and is dependent on the daily surges of gonadotropins (2). Furthermore, we have demonstrated that advancing or delaying the day of initiation of the daily surges correspondingly advances or delays the age of puberty (3). Neonatal androgenization, which renders the adult permanently acyclic, also abolishes the prepubertal daily surges of LH and FSH (4). In sum, these results support the proposal (2) that the daily surges of gonadotropins may regulate in some manner the rate of sexual maturation and thus age at puberty.

Development of the hypothalamus–pituitary–gonad axis involves multiple, interdependent processes (5), and significant questions remain about central mechanisms that determine the age of puberty. A restraining influence of the central nervous system independent of sex steroids has been proposed (6). A plausible mechanism via endogenous opioid inhibition of pubertal development is suggested on the basis of experiments which show that naloxone (NAL) or other opiate receptor antagonists rapidly increase serum levels of LH (7, 8) while morphine, a receptor agonist, depresses basal serum LH levels (9).

Since experimental advancement of the age of onset of cyclical LH release will lead to an early onset of puberty in the female hamster (3) and since NAL induces the release of LH in the immature female rat, it seemed plausible that chronic NAL administration to the very young female hamster might advance initiation of endogenously generated cyclic release of LH. We would predict that as a consequence, puberty would be correspondingly advanced. Such data would provide evidence of a specific mechanism by which a restraining influence of the central nervous system might be expressed. The results of experiments presented here suggest that while NAL advances the age of puberty, it does so by directly stimulating release of gonadotropins rather than



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by advancing the age of endogenously induced gonadotropin surges.

**Materials and Methods.** The Syrian hamsters (*Mesocricetus auratus*) used in this study were produced in the Delaware colony. In all cases, they were maintained on L:D 14:10 (lights on at 0600 hr) and weaned at 21 days of age at which time the individuals were isolated from males and held in groups of similar size. The animals were sorted by pelage color at this time so that records could be maintained for each individual. They were provided free access to food and water.

*Experiment I: Effect of NAL on age of first vaginal discharge.* Naloxone (gift of E. I. DuPont de Nemours & Co., Wilmington, Del.) was injected daily (sc, 50 mg/kg body wt, dissolved in sterile isotonic saline) for 17 days, beginning on the day of birth. Injections were given between 1300 and 1400 hr and controls received an equivalent volume of vehicle. Each individual was checked daily between 13 and 18 days of age for eye opening. After weaning at 21 days of age, animals were examined daily for appearance of the vaginal discharge that occurs after spontaneous ovulation in the adult (10). Thus, the first of these characteristic discharges is evidence that puberty has occurred, and first vaginal discharge is an index of puberty. To verify that regular estrous cycles had been initiated, we continued our examinations until three consecutive discharges had occurred at 4-day intervals.

*Experiment II: Effect of NAL on endogenously generated LH surges.* Naloxone was injected between 1200 and 1300 hr. Age at eye opening was determined and, in addition, groups were sacrificed on 14, 15, 16, 17, and 18 days of age at 1400, 1700, and 2000 hr. Blood samples collected at these times allowed us to determine if a daily surge had occurred since maximum levels occur at 1700 hr and thus determine if effects of NAL on age of first vaginal discharge are mediated through an early initiation of daily surges of gonadotropin release. If NAL elicits release of LH, surges induced by injections before 1300 hr should not affect the detection of a peak of serum LH at 1700 hr. Two additional groups of naloxone- and saline-injected females were checked for age at first vaginal discharge.

*Experiment III: Does NAL injection elicit an LH surge?* We first determined the time course of the LH response, if any, by sacrificing 22-day-old females at intervals between 15 and 120 min after injection of NAL or saline; subsequently, to assess the effect of NAL on the release of LH during the period of injections of Experiments I and II, and acute response was determined also at 14 days of age. LH levels were measured in blood samples collected 30 min after administration of several different doses of NAL to 14-day-old females. In these studies, NAL injections were given at 1100 hr.

*Assays and statistics.* LH was measured by a double antibody RIA procedure using materials provided by NIADDK for the measurement of rat LH. Serum LH values were expressed in terms of rat LH-RP-1. The serum samples from Experiment II were assayed using rLH-RP-1 as the reference preparation and anti-rLH-S-6 as the antiserum. Samples from Experiment III were assayed using rLH-RP-2 and the reference preparation and anti-rLH-S-8 and the antiserum. In both cases, the iodinated preparation was rLH-I-6. A pool of hamster serum samples measures 11.5× greater in the assays with rLH-RP-1 as the reference preparation and the values of samples in Experiment III were so adjusted and expressed as RP-1 for consistency. The intraassay coefficient of variation was 16.3 and 11.9%, respectively, for the RP-1 and the RP-2 systems.

The Mann–Whitney nonparametric test was used to determine the significance of differences in age of eye opening and first vaginal discharge, and one-way ANOVA, followed by the Student–Newman–Keuls test where appropriate, was used for LH data.

**Results.** Naloxone, in both Experiments I and II, significantly advanced the first day of observable vaginal discharges 6 days (Table I) as compared to saline-injected controls. That this effect was not elicited by advancement of the initiation of endogenous daily surges of LH is shown in Fig. 1, in which both saline- and naloxone-injected animals initiated cyclical surges of LH release at 16 days of age. However, NAL injections did induce release of LH in 14- and 22-day-old females with a maximum occurring 30 min after the injections (Fig. 2). A similar release of LH was induced by NAL injections that were at least 10-fold more dilute (Table II) than the ones used

TABLE I. THE EFFECT OF DAILY NALOXONE INJECTIONS ON AGE OF FIRST VAGINAL DISCHARGE

| Group | Days of age | |
|---|---|---|
| | Experiment I | Experiment II |
| Saline | 38 ± 1 (14)[a] (range 29–44) | 37 ± 1 (42) (range 29–48) |
| Naloxone | 32 ± 1 (22)* (range 24–39) | 31 ± 1 (51)* (range 23–46) |

[a] Mean ± SEM (*n*).
* $P < 0.001$ vs saline.

routinely herein. There were no consistent discernable effects of NAL injections on the age of eye opening (averaged 16 or 17 days of age in both saline- and naloxone-injected animals in both Experiments I and II) or on body weight (means of 18.5 g vs 17.2 g saline- vs naloxone-injected on Day 17 in Experiment I, $P < 0.4$, unpaired *t* test).

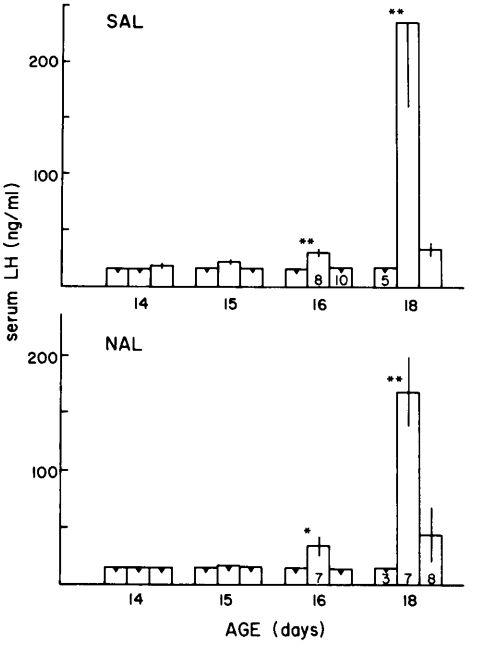


FIG. 1. Onset of daily surges of LH in immature female hamsters. The first, second, and third bars in each group represent samples collected at 1400, 1700, and 2000 hr, respectively. Except where noted, there were six saline-injected animals and eight naloxone-injected animals per group. The dose of naloxone was 50 mg/kg body wt and injections were given between 1200 and 1300 hr. All data are presented as means + SEM. Inverted triangles represent groups in which the levels in most animals were below the sensitivity of the assay. *$P < 0.05$ vs 1400 hr; **$P < 0.01$ vs 1400 hr.

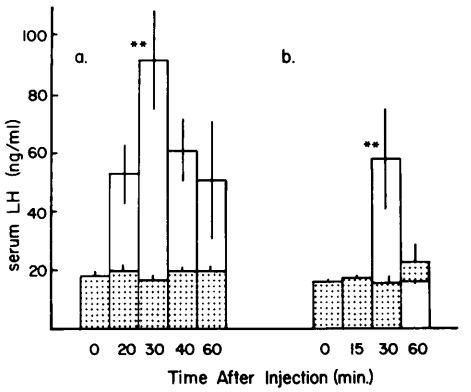


FIG. 2. Serum LH levels after injection of naloxone (50 mg/kg body wt, open bars) or saline (stippled bars) at 1100 hr. (a) Fourteen-day old-animals. Eight animals per group in the naloxone-injected, except at 60 min when six animals were available, and six animals per saline-injected group. (b) Response to naloxone at 22 days of age; five animals per group. Data are pesented as means ± SEM. **$P < 0.01$ vs 0 group.

**Discussion.** In both Experiments I and II, single, daily injections of NAL from Days 1 through 17 of age advanced first vaginal estrus discharge by an average of 6 days as compared to saline-injected controls. These results suggest that first ovulations (puberty) occurred earlier in the naloxone-treated animals than in the saline-injected animals. One should be cautious about inferring that puberty is coincident with first vaginal discharge in either group, however. We have found (R. S. Donham and M. H. Stetson, unpublished) that ova

TABLE II. LH LEVELS 30 min AFTER INJECTION OF VARIOUS DOSES OF SALINE OR NALOXONE

| Dose (mg/kg body wt) | LH (ng/ml serum) |
|---|---|
| Saline | 15.1 ± 1.3 (6)[a] |
| 0.005 | 15.4 ± 1.3 (6) |
| 0.05 | 15.1 ± 1.2 (6) |
| 0.5 | 25.7 ± 3.2 (6)* |
| 5.0 | 62.9 ± 11.3 (5)* |
| 50.0 | 70.2 ± 14.4 (6)* |

[a] Mean ± SEM (*n*).
* $P < 0.01$ vs saline.

are invariably found in the oviducts of females with characteristic vaginal discharges. Similarly, Diamond and Yanagimachi (11) found that while first ovulations (as determined by laparotomy after vaginal estrus) in pubertal female hamsters were not always preceded by the behavioral manifestations of estrus, oviductal ova were always present if a vaginal discharge was observed. The possibility remains, however, that undetected ovulations could have occurred before the first vaginal discharge. Even if this were so, our argument that puberty occurred earlier in the naloxone-injected females remains unaffected unless there is a difference between naloxone-treated and saline-injected animals with regard to the delay between possible "silent" ovulations and the first vaginal discharge.

Naloxone probably advances pubertal age by enhancing LH release. Naloxone induced a surge of LH at both 14 and 22 days of age and has been demonstrated to have the same effect in both immature and adult female rats (7, 8). The results of Experiment III show that similar effects might be expected from lesser doses of NAL (Table II).

At the same time there was no evidence of an effect on the neural maturation processes required to support the initiation of daily cycles of LH release since the onset of endogenous surges was similar in both naloxone- and saline-treated animals (Fig. 1). Daily administration of the short-acting antagonist before 1300 hr elicited a spike in peripheral LH levels which decayed by 1500 hr or so, thus assuring that the induced spikes would not be confused with the endogenously generated ones occurring at 1700 hr. The finding that the age of eye opening was similar in both groups also suggests that advancement of puberty is not correlated with early neuronal maturation. Thus, under the limited conditions of this experiment, it does not appear that NAL influences endogenous LH surges, but the results do not preclude the possibility that other drugs and/or drug regimens might uncover such an effect.

We think it most likely that an injection-induced increase in circulating LH resulted in a daily stimulation of ovarian development and an advancement of pubertal age. In the hamster, maturation processes occurring in the ovaries determine, at least in part, the rate of sexual maturation (R. S. Donham and M. H. Stetson, unpublished). When daily surges of LH and FSH begin, normally at about 16 or 17 days of age, endocrine and follicular development of the ovaries accelerates. This results in follicles that are ovulable at about 26 to 28 days of age (12) and progesterone levels that approach those of the adult (2). Early initiation of daily release of LH, whether by NAL or by exogenous gonadotropin-releasing hormone (GnRH), as shown previously (3), apparently advances the development of the ovaries so that adult gametogenic and endocrine function is attained about 6 days earlier. The reason that NAL injections, initiated on the day of birth, do not advance the age of first vaginal discharge even further than the 6 days observed may be a result from unresponsiveness of the hamster pituitary to GnRH prior to Day 8 of age (13) and from lack of gonadotropin receptors in the ovaries prior to 10 to 15 days of age (14).

While this work was in progress it was reported that chronic NAL administration from Days 1 and 10 of age induced precocious puberty in the female rat (15). Thus, in rodents, accumulating evidence suggests that exogenous opiate antagonists may accelerate developmental processes in early stages of sexual maturation with chronologically remote effects. We suggest, however, that these results do not necessarily mean that there is an endogenous opiate influence that restrains the pubertal process. In the immature female hamster, if endogenous opiates can be shown to inhibit initiation of daily rhythms of gonadotropin release, an event that appears to be crucial to the rest of sexual maturation (2–4), the argument that age at puberty is regulated by activity of opiate system would be supported. Our results, demonstrating the lack of an effect of NAL on initiation of cyclic LH release and the absence of an effect on eye opening, suggest that the puberty-advancing effect of the antagonist may be pharmacologic.

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1. Smith SG, Stetson MH. Maturation of the clock-timed gonadotropin release mechanism in hamsters: A key event in the pubertal process? Endocrinology (Baltimore) **107**:1334–1337, 1980.
2. Donham RS, DiPinto MN, Stetson MH. Twenty-four hour rhythms of gonadotropin release induces cyclic progesterone secretion by the ovary of prepubetal and adult hamsters. Endocrinology (Baltimore) **114**:821–826, 1984.
3. Donham RS, Creyaufmiller N, Lyons TJ, Stetson MH. Temporal relationship between the onset of daily gonadotrophin surges and of puberty in female golden hamsters. J Endocrinol, **108**:219–224, 1986.
4. Donham RS, Stetson MH. Neonatal androgen abolishes clock-timed gonadotropin release in prepubertal and adult female hamsters. J Reprod Fertil **73**:215–221, 1985.
5. Ojeda SR, Aguado LI, Smith (White) S. Neuroendocrine mechanisms controlling the onset of female puberty: The rat as a model. Neuroendocrinology **37**: 306–313, 1983.
6. Reiter EO, Grumbach MM. Neuroendocrine control mechanisms and the onset of puberty. Annu Rev Physiol **44**:595–613, 1982.
7. Blank MS, Panerai AE, Friesen HG. Opioid peptides modulate luteinizing hormone secretion during sexual maturation. Science **203**:1129–1131, 1979.
8. Schulz R, Wilhelm A, Pirke KM, Herz A. Regulation of luteinizing hormone secretion in prepubertal male and female rats. Life Sci **31**:2167–2170, 1982.
9. Van Vugt DA, Meites J. Influence of endogenous opiates on anterior pituitary function. Fed Proc **39**:2533–2538, 1980.
10. Orsini MW. The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation and the anestrous hamster, *Mesocricetus auratus* (Waterhouse). Proc Anim Care Panel **11**:193–206, 1961.
11. Diamond M, Yanagimachi R. Reproductive development in the female golden hamster in relation to spontaneous estrus. Biol Reprod **2**:223–229, 1970.
12. Greenwald GS, Peppler RD. Prepubertal and pubertal changes in the hamster ovary. Anat Rec **161**:447–458, 1968.
13. Smith SG, Matt KS, Prestowitz WF, Stetson MH. Regulation of tonic gonadotropin release in prepubertal female hamsters. Endocrinology (Baltimore) **110**: 1262–1267, 1982.
14. Shaha C, Greenwald GS. Development of steroidogenic activity in the ovary of the prepubertal hamster. I. Response to in vivo or in vitro exposure to gonadotropins. Biol Reprod **28**:1231–1241, 1983.
15. Sirinathsinghji DJS, Motta M, Martini L. Induction of precocious puberty in the female rat after chronic naloxone administration during the neonatal period: The opiate "brake" on prepubertal gonadotrophin secretion. J Endocrinol **104**:299–307, 1985.

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