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Naltrexone Effects on Pituitary and Gonadal Hormones in Male and Female Rhesus Monkeys

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MELLO, N. K., J. H. MENDELSON, M. P. BREE AND A. SKUPNY. *Naltrexone effects on pituitary and gonadal hormones in male and female rhesus monkeys.* PHARMACOL BIOCHEM BEHAV 31(3) 683–691, 1988.—The long-acting opioid antagonist, naltrexone, stimulates LH and FSH in women during the early follicular phase of the menstrual cycle and is a new provocative test of hypothalamic-pituitary function (42,63). The acute effects of naltrexone (0.25, 0.50 and 1.0 mg/kg IV) on anterior pituitary (LH, FSH, PRL) and gonadal steroid (T or E_2) hormones were studied in 7 female and 4 male rhesus monkeys (*Macaca mulatta*). Integrated plasma samples were collected at 20 min intervals for 60 min before and for 300 min after intravenous infusion of naltrexone over 10 min. In females studied during the early follicular phase (cycle days 1–3), naltrexone did not stimulate LH and significantly suppressed E_2 ($p<0.0003$–0.0001) and FSH ($p<0.006$–0.0001). Naltrexone (0.50 and 1.0 mg/kg) also did not stimulate LH release in late follicular phase females (cycle days 10–12) when estradiol levels were in the peri-ovulatory range. FSH and E_2 were significantly suppressed ($p<0.01$–0.05) after 1.0 mg/kg naltrexone, but not after 0.5 mg/kg naltrexone. However, in males all doses of naltrexone significantly stimulated LH ($p<0.003$–0.0001) and T ($p<0.001$–0.0001) but not FSH. LH increased significantly above baseline within 20 to 40 min and T increased significantly within 60 min. These gender differences in naltrexone's effects on pituitary gonadotropins and gonadal steroid hormones were unanticipated. These data are not concordant with clinical studies which report significant naltrexone stimulation of LH in men and in women during the early follicular phase. Only prolactin levels decreased after naltrexone infusion in both males and females and this decrease was statistically significant after a dose of 0.25 mg/kg in males ($p<0.008$) and after 0.50 and 1.0 mg/kg in females ($p<0.0001$).

Naltrexone Opioid antagonists Gonadotropins, naltrexone effects on
Gonadal steroid hormones, naltrexone effects on Naltrexone effects, gender differences in

IT is well known that opiates, such as morphine or heroin, suppress gonadotropin release (11,12) and it now appears that the inhibitory regulation of hypothalamic luteinizing hormone-releasing hormone (LHRH) is mediated by endogenous opioids (17,72). Opioid antagonist drugs rapidly stimulate pituitary gonadotropin release, presumably by antagonism of endogenous opioid peptide inhibitory effects (20, 40, 42, 45, 47, 72). The short-acting opioid antagonist, naloxone, reliably increases plasma luteinizing hormone (LH) levels during the late follicular and luteal phases of the menstrual cycle in women (4, 41, 58, 61, 72) and in female rhesus monkeys (54, 66, 67). However, naloxone does not stimulate pituitary gonadotropins during the early follicular phase of the menstrual cycle (41, 54, 58, 61, 66, 67, 72).

Naloxone's ineffectiveness in stimulating LH during the early follicular phase is usually attributed to low basal levels of ovarian steroid hormones (17,72), but the short duration of naloxone's opioid antagonist action also may be a contributing factor. The half-life of naloxone in plasma is about 1 hr and the duration of its opioid antagonist action is between 1 and 4 hr after parenteral administration (27). In contrast, the long-acting opioid antagonist, naltrexone, has a half-life of 10 hr in plasma and its opioid antagonist actions may persist for as long as 24 hr (27,68).

Unlike naloxone, naltrexone stimulates pituitary release of LH, follicle stimulating hormone (FSH), prolactin (PRL) and adrenocorticotropic hormone (ACTH) during the early follicular phase (days 2–4) of the menstrual cycle in normal women (42,63). Chronic naltrexone administration has been shown to induce ovulation in women with secondary hypothalamic amenorrhea (69). Discontinuation of daily naltrexone administration (50 mg PO) was followed by a recrudescence of amenorrhea, associated with abnormally low levels of pituitary gonadotropins and estradiol (E_2) (69).

Naltrexone is a potentially useful provocative test for analyzing the consequences of chronic drug abuse on hypothalamic-pituitary function (42,63). It is well known that chronic abuse of many substances (marihuana, opiates, cocaine, alcohol) may cause profound disruptions of reproductive function (6, 11, 12, 15, 35). For example, chronic alco-

holism is associated with amenorrhea, luteal phase dysfunction and anovulation in women (26, 35, 48, 62, 65) and with low testosterone (T) levels, testicular atrophy, gynecomastia and impotence in men (5, 13, 52, 65), but the site or sites of alcohol's toxicity remain to be determined. The challenge for analysis of alcohol and other abused drug effects on the hypothalamic-pituitary-gonadal axis has been to devise techniques to dissect out and evaluate the effects of alcohol and drugs on each component of this system separately (35). Synthetic LHRH is an effective test of pituitary function (38, 39, 59, 71) and human chorionic gonadotropin (hCG) is one provocative test of ovarian function (50,59). Naltrexone and other opioid antagonist drugs are thought to directly stimulate hypothalamic release of endogenous LHRH which, in turn, stimulates pituitary gonadotropin secretory activity (72).

The present study is an evaluation of the efficacy of naltrexone, a long-acting opioid antagonist, as a gonadotropin stimulus in male and female rhesus monkeys (*Macaca mulatta*). This primate model is used widely in studies of reproductive function because neuroendocrine regulation of the hypothalamic-pituitary-gonadal axis is very similar to humans (22, 28, 29, 51). Both naltrexone and naloxone stimulate LH in human males (40, 45–47, 49). Naloxone also stimulates LH in male Macaque monkeys (19,36) and naltrexone has been reported to increase LH and T with a concomitant inhibition of sexual behavior in normal male rhesus and talapoin monkeys (1,34). We are unaware of any previous studies of naltrexone's effects on anterior pituitary and gonadal steroid hormones in female rhesus monkeys. The effects of naltrexone on anterior pituitary hormones were compared in normal males and normal females during both the early and late follicular phase of the menstrual cycle.

METHOD

Subjects

Seven normally cycling adult rhesus females and four normal adult rhesus males (*Macaca mulatta*) were studied. The males weighed 8.2±0.68 kg and the females weighed 6.7±0.41 kg. Females with normal ovulatory menstrual cycles were studied on two occasions, during the early follicular phase of the menstrual cycle (cycle days 1 to 3) and during the late follicular phase (cycle days 10 to 12).

All monkeys lived in individual cages and a twelve hr light/dark cycle (7 a.m. to 7 p.m.) was in effect. Monkey chow was supplemented with fresh fruit, vegetables and multiple vitamins. Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources and protocols were approved by the Institutional Animal Care and Use Committee. The facility is licensed by the Department of Agriculture. The health of the monkeys was monitored periodically by a consultant veterinarian from the New England Regional Primate Research Center.

Sequence of Conditions

The acute effects of naltrexone on anterior pituitary and gonadal hormones were evaluated. Basal levels of LH, FSH, PRL, and T or E_2 were measured for 60 min (three 20-min samples) before naltrexone infusion, then for an additional 300 min (fifteen 20-min samples) following naltrexone administration. Naltrexone HCl (0.25, 0.50 and 1.0 mg/kg) diluted in sterile saline was infused into the saphenous vein of the leg opposite the blood sample exfusion catheter over an interval of 10 min. Naltrexone doses were given in an irregular order (0.50 mg/kg, 0.25 mg/kg, 1.0 mg/kg) and an interval of at least one menstrual cycle (or 30 days in males) intervened between successive study days.

Integrated Plasma Sample Collection Procedures

Since pituitary gonadotropins are secreted episodically (28–30) we developed an acute venous catheterization procedure for integrated plasma sample collection (7). An integrated plasma sample collection procedure was used in preference to a discrete bolus sample collection method, since a bolus sample might coincide with either the peak or the nadir of episodic pituitary secretory activity.

Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg IM) and the saphenous vein was catheterized with a 22 g Deseret® Radiopaque Intracath (Deseret Medical, Inc., Park Davis, Co., Sandy, VT) using aseptic procedures. The intracath was connected to a sterile, heparin-soaked silicone tube and secured with sutures. Ketamine was used because it does not effect the release of LH, gonadotropin releasing hormone (GnRH) and PRL in rhesus monkey (16, 18, 56). After catheterization, the monkey was placed in a standard primate chair and sample collection began within 30 min. Blood was exfused continuously with a Rainin Rabbit Miniature Peristaltic Pump (Rainin Instrument Co., Inc., Woburn, MA) into heparinized vacutainer tubes in chipped ice. Blood samples were collected at 15 or 20 min intervals, centrifuged and aliquots of plasma were withdrawn and frozen at −70°C.

Luteinizing Hormone (LH) Radioimmunoassay

Plasma LH concentrations were determined in duplicate by a double-antibody radioimmunoassay procedure similar to that described by Midgley (43) using materials prepared by Dr. W. Peckham and following his suggestions. Purified ceropithecus pituitary LH for radioiodination (WP-XV-117-3239), rabbit antiserum (R13, pool D) to hCG and rhesus pituitary LH reference preparation (NICHD-rhLH, also known as WP-XV-20) were provided by the National Hormone and Pituitary Program supported by the National Institute of Child Health and Human Development and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Radioiodination was carried out using the chloramine-T method (24) with sodium iodide-125 purchased from DuPont/NEN Products (Billerica, MA) Goat antirabbit gamma globulin was obtained from Behring Diagnostics (San Diego, CA). Results are expressed as ng/ml in terms of the reference preparation. The assay sensitivity was 7.3 ng/ml. Intra- and interassay coefficients of variation were 5.6 and 12.2 percent, respectively.

Follicle Stimulating Hormone (FSH) Radioimmunoassay

Plasma FSH concentrations were determined in duplicate by a double antibody radioimmunoassay similar to that described by Midgley (44) using materials intended for macaque and baboon FSH (25). Antiserum to human FSH (Batch No. 5, prepared in rabbits by A. F. Parlow) and rhesus pituitary gonadotropin reference preparation (cyn-FSH-RP-1, prepared by Dr. L. E. Reichert) were provided by the National Hormone and Pituitary Program, supported by the National Institute of Child Health and Human Devel-

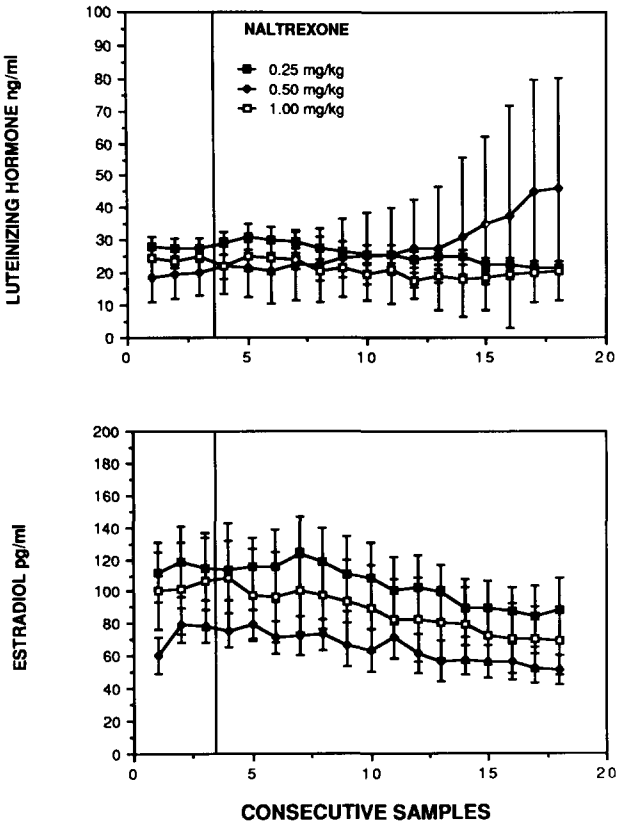

FIG. 1. Naltrexone effects on LH (nanograms per milliliter) and E_2 (picagrams/ml) in rhesus females during the early follicular phase. Each LH data point is the mean±S.E. of 4 or 5 subjects; each E_2 data point is the mean±S.E. of 5 or 6 subjects. Integrated plasma sample values for LH and E_2 are shown for three consecutive 20 min samples before IV naltrexone administration (0.25, 0.5 or 1.0 mg/kg) and for 15 consecutive 20-min samples after naltrexone administration.

opment and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Iodine-125-labeled human FSH was purchased from Cambridge Medical Diagnostics (Billerica, MA). Goat antirabbit gamma globulin was obtained from Behring Diagnostics (San Diego, CA). Results are expressed as ng/ml in terms of the reference preparation. The assay sensitivity was 1.6 ng/ml and the intra- and interassay coefficients of variation were 14.2 and 23.7 percent, respectively.

Prolactin (PRL) Radioimmunoassay

Plasma PRL concentrations were determined by a double antibody radioimmunoassay designed for analysis of human PRL using a procedure similar to that described by Midgley (43). Human PRL antiserum (NIADDK-anti-hPRL-3) and reference preparation (NIADDK-hPRL-RP-1) were provided by the National Hormone and Pituitary Program, supported by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Iodine-125-labeled human PRL was purchased from Cambridge Medical Diagnostics (Billerica, MA). Goat antirabbit gamma globulin was obtained from Behring Diagnostics (San Diego, CA). Results are expressed as ng/ml in terms of the reference preparation. The assay sensitivity was 5 ng/ml. Intra- and interassay coefficients of variation were 4.7 and 5.3 percent, respectively.

Testosterone (T) Radioimmunoassay

Plasma T concentrations were measured in duplicate by a direct double antibody radioimmunoassay method that did not require solvent extraction, using a kit purchased from Radioassay Systems Laboratories (Carson, CA). Results are expressed as ng/dl. Assay sensitivity was 4 ng/dl. Intra- and interassay coefficients of variations were 3.2 and 4.8 percent, respectively.

Estradiol (E_2) Radioimmunoassay

Plasma E_2 concentrations were measured in duplicate by a double antibody radioimmunoassay method with extraction, using a kit purchased from Serono Diagnostics, Inc. (Norwell, MA). Results are expressed as pg/ml 17-beta-estradiol. Assay sensitivity was 1.7 pg/ml. Intra- and interassay coefficients of variation were 7.9 and 9.6 percent, respectively.

Statistical Analysis

The effects of naltrexone on pituitary and gonadal hormones and cortisol were evaluated with a two-way Analysis of Variance (ANOVA) for repeated measures. Baseline control samples 1–3 were compared with postnaltrexone samples 4–18. If ANOVA showed a significant main effect, Tukey's W follow-up tests were used (55). A one-way ANOVA for repeated measures was also run for each dose group and followed with Dunnett's t-test for comparison of multiple experimental groups with a single control group (70). Dunnett's t-test was used to compare group mean values at each postnaltrexone sample period with the baseline mean hormone values. In some instances, data are presented as the algebraic difference from the pretreatment baseline (i.e., delta change or difference scores).

RESULTS

Naltrexone Effects on LH and Estradiol in Normal Females

Naltrexone did not stimulate LH in females studied during the early follicular phase (Fig. 1). LH levels before administration of 0.25, 0.50, and 1.0 mg/kg naltrexone averaged 28±1.6, 19±3.9 and 24±0.7 ng/ml. Baseline LH levels prior to administration of 0.25 mg/kg naltrexone were significantly higher than before administration of 0.50 mg/kg naltrexone ($p<0.01$). LH decreased significantly ($p<0.05$) 240 min after administration of 0.25 mg/kg naltrexone. LH increased gradually within 280 min after administration of 0.50 mg/kg naltrexone (sample 17) but this change was not statistically significant (Fig. 1).

Estradiol levels decreased significantly after each dose of naltrexone ($p<0.0001$ to 0.0003) (Fig. 1). Baseline E_2 levels averaged 115±20, 73±9.7, and 103±27 pg/ml before the low, moderate and high doses of naltrexone. Estradiol levels fell significantly below baseline levels within 160 to 200 min following naltrexone administration ($p<0.05$ to 0.01) (samples 11–13) (Fig. 1).

Naltrexone Effects on LH and T in Males

Baseline LH levels (samples 1–3) were equivalent and averaged 24±4.6 ng/ml, 20±3.1 ng/ml and 28±4.5 ng/ml be-

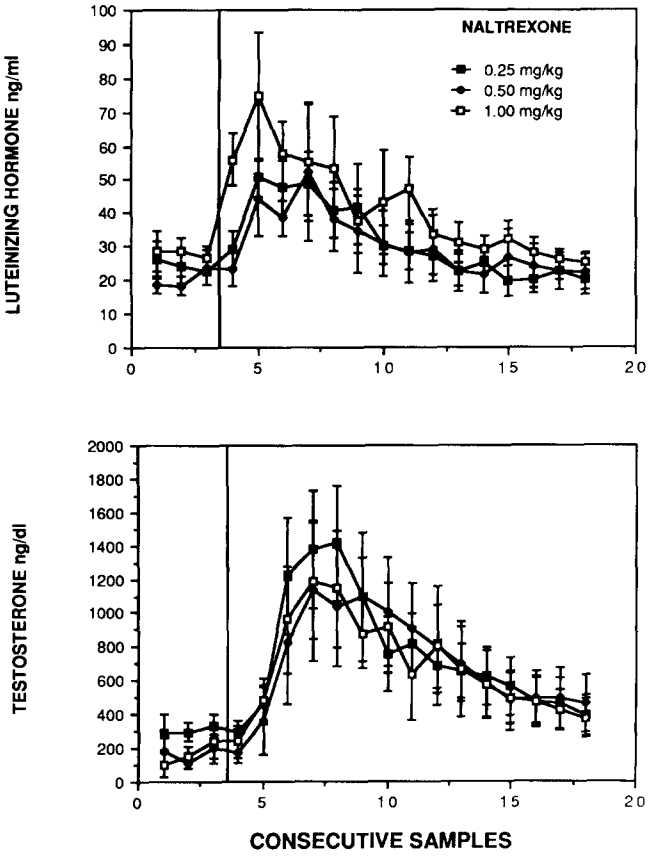

FIG. 2. Naltrexone effects on LH (nanograms per milliliter) and testosterone (nanograms per deciliter) in male rhesus monkeys. Each data point is the mean±S.E. of four subjects. Integrated plasma sample values for LH and T are shown for three consecutive 20 min samples before IV naltrexone administration (0.25, 0.5 or 1.0 mg/kg) and for 15 consecutive 20-min samples after naltrexone administration.

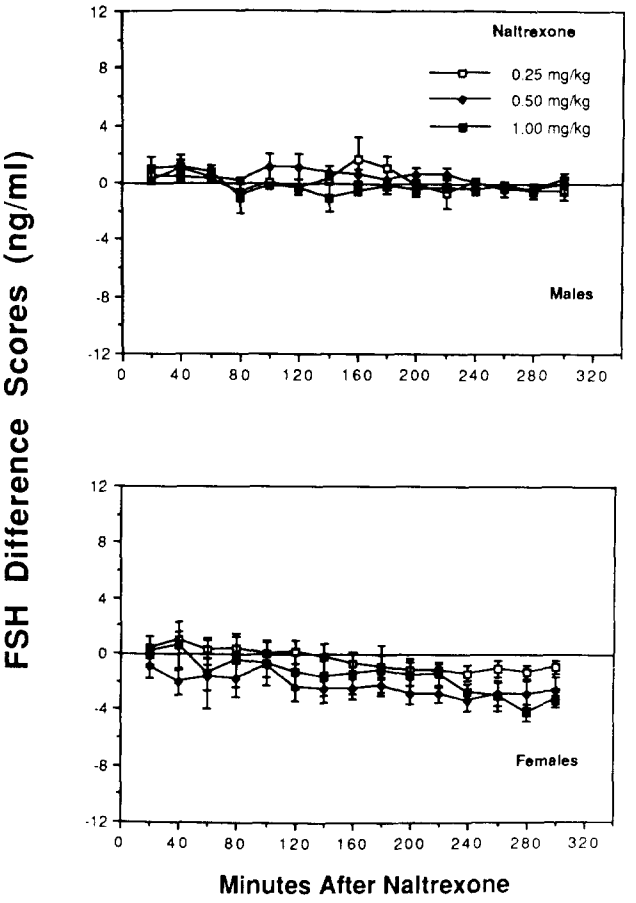

FIG. 3. Naltrexone effects on FSH in rhesus males and females during the early follicular phase expressed as difference scores from the prenaltrexone baseline levels. Each FSH data point is the mean±S.E. of 4 or 5 subjects. Time after IV naltrexone administration (0.25, 0.5 or 1.0 mg/kg) is shown on the abscissa.

fore administration of 0.25, 0.50 and 1.0 mg/kg naltrexone. In contrast to data obtained in females, naltrexone stimulated a significant increase in LH within 20 to 40 min at all doses ($p<0.003$–0.0001) (Fig. 2, top). LH levels remained significantly above baseline for 80 to 100 min. After administration of 0.25, 0.50 and 1.0 mg/kg of naltrexone, LH increased to 109, 158 and 167 percent above baseline. Although the highest dose of naltrexone (1.0 mg/kg) produced the greatest increase in LH, this was not significantly greater than LH peaks after lower doses of naltrexone according to ANOVA.

Baseline levels of T averaged 307±63, 164±57 and 164±71 ng/dl before naltrexone (0.25, 0.50 and 1.0 mg/kg) administration. Testosterone levels increased significantly within 60 min (sample 6) after each dose of naltrexone ($p<0.001$–0.0001) and reached peak levels within 80 to 100 min (samples 7 and 8) (Fig. 2, lower panel). Naltrexone-stimulated T remained significantly elevated over baseline levels for 120 to 180 min (samples 9 to 12). Peak levels of T occurred 60 min after peak LH levels following 0.25 mg/kg naltrexone; simultaneously with peak levels of LH following 0.50 mg/kg naltrexone; and 40 min after the LH peak following 1.0 mg/kg naltrexone.

Naltrexone Effects on FSH in Males and Females

In males, there was no systematic pattern of changes in FSH after any dose of naltrexone. FSH averaged 3±2, 2.4±1.5 and 3±1.6 ng/ml before administration of 0.25, 0.50 and 1.0 mg/kg naltrexone. FSH did not change significantly following naltrexone administration according to ANOVA. These data are shown as difference scores in Fig. 3 (top).

In early follicular females, baseline FSH levels were 3.4±0.54, 5±0.98 and 7.9±1.9 ng/ml before administration of a low, moderate and high dose of naltrexone. FSH decreased significantly after all doses of naltrexone ($p<0.006$ to 0.0001). After 0.25 mg/kg of naltrexone, FSH was significantly depressed within 200 min ($p<0.05$). A more rapid (40 min) and sustained suppression of FSH occurred after administration of 0.50 mg/kg naltrexone ($p<0.05$–0.01). After 1.0 mg/kg naltrexone, FSH was significantly suppressed within 240 min.

Naltrexone Effects on Prolactin in Males and Females

Prolactin levels in males averaged 20±6.5, 22±6.8 and 26±7.6 ng/ml before naltrexone administration. Administra-

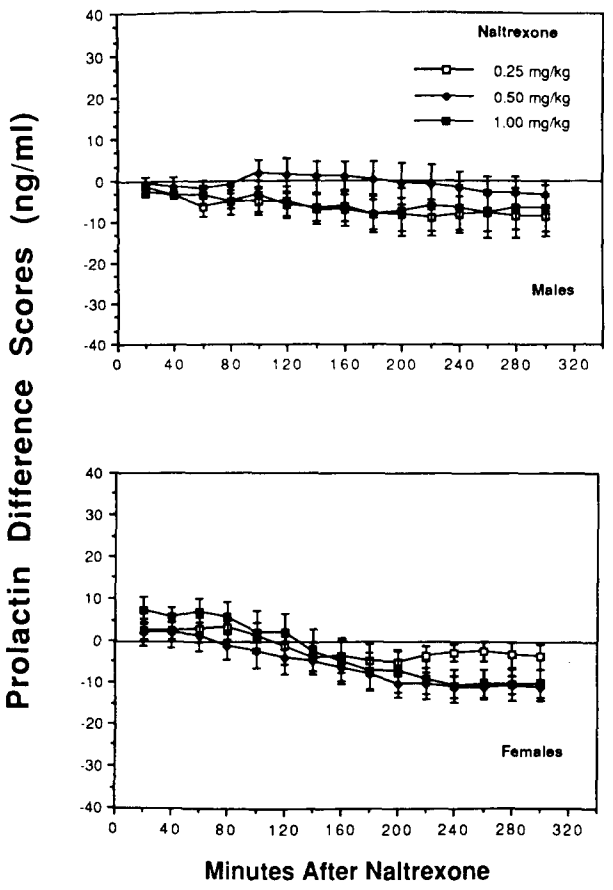

FIG. 4. Naltrexone effects on PRL in males and females expressed as difference scores from the prenaltrexone baseline levels. Each data point is the average of 4 male monkeys and 5 or 6 females in the early follicular phase. Time after IV naltrexone administration (0.25, 0.5 or 1.0 mg/kg) is shown on the abscissa.

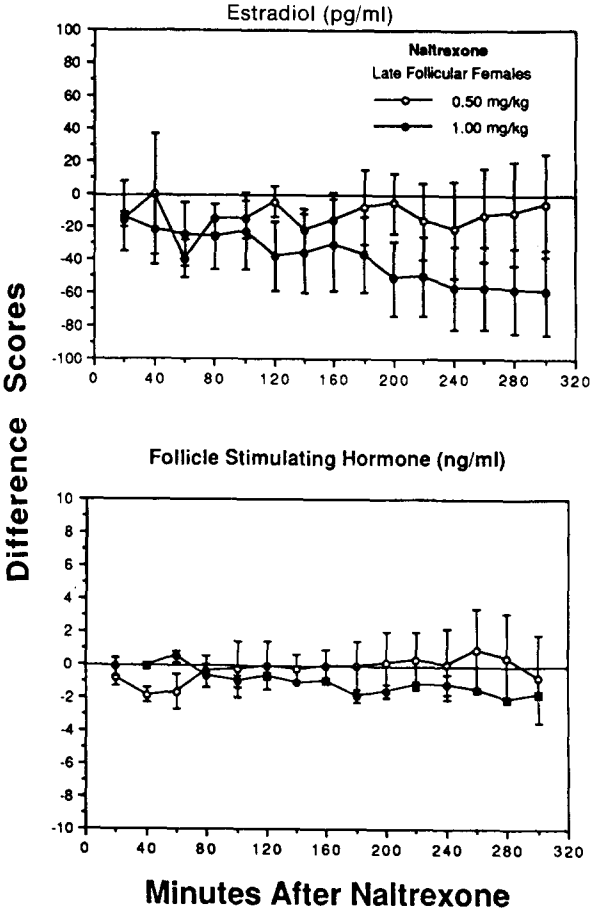

FIG. 5. Naltrexone effects on FSH (bottom) and estradiol (top) expressed as difference scores from the prenaltrexone baseline levels. Each data point is the average of 3 female monkeys in the late follicular phase (day 10–12). Time after IV naltrexone administration (0.5 or 1.0 mg/kg) is shown on the abscissa.

tion of 0.25 mg/kg naltrexone significantly suppressed PRL levels ($p<0.008$) within 60 to 120 min (samples 6–12). Administration of 0.50 and 1.0 mg/kg of naltrexone was followed by a nonsignificant decrease in PRL. These data are shown in Fig. 4 (top) as difference scores.

In early follicular phase females' baseline PRL levels were 18±3.2, 21±4.1 and 27±2.7 ng/ml prior to low, moderate and high doses of naltrexone. All doses of naltrexone were followed by a gradual decline in PRL (Fig. 4, lower panel). The lowest dose of naltrexone did not decrease PRL significantly below baseline, but the moderate and high naltrexone doses decreased PRL significantly ($p<0.0001$). Prolactin levels fell significantly below baseline within 160 min after 0.50 mg/kg naltrexone and within 220 min after 1.0 mg/kg naltrexone ($p<0.05$ to 0.01).

Naltrexone Effects in Normal Females During the Late Follicular Phase

Naltrexone (0.50 and 1.0 mg/kg) also did not stimulate LH in females studied on days 10–12 of the menstrual cycle (data not shown). Baseline LH levels averaged 28±2.5 ng/ml. The effects of naltrexone on FSH and E_2 were dose-dependent. The 0.50 mg/kg dose of naltrexone did not change E_2 or FSH significantly from baseline levels of 162±78 pg/ml and 6±1 ng/ml. However, after 1.0 mg/kg of naltrexone, E_2 was significantly depressed ($p<0.01$–0.05) within 120 minutes and remained suppressed throughout the 300 min sampling period (Fig. 5). FSH also decreased significantly after naltrexone administration (1.0 mg/kg) and remained suppressed throughout the sampling period (Fig. 5).

Naltrexone induced significant ($p<0.0001$) but inconsistent changes in PRL in late follicular females according to ANOVA. Prolactin levels decreased significantly ($p<0.01$) within 120 min after administration of 0.50 mg/kg naltrexone. But after 1.0 mg/kg naltrexone, PRL increased significantly within 20 min and remained elevated (38–39 ng/ml) for 100 min ($p<0.01$–0.05). Prolactin subsequently fell below baseline levels (28±1.3 ng/ml) but these changes were not significant (Fig. 5).

DISCUSSION

Naltrexone Effects on LH, FSH and E_2 in Females

The absence of an LH response to naltrexone in rhesus females studied during the early follicular phase (Fig. 1) is at variance with previous studies in human females. Nal-

trexone (50 mg/PO) significantly increased LH, PRL, ACTH and cortisol in normal women during the early follicular phase (cycle days 1–4) (42). Replication of this study in 14 women during cycle days 2–4 (63) attests to the robustness of naltrexone stimulation during the early follicular phase in human females. Naltrexone administration also stimulates LH during the midluteal phase in women (Mendelson, J. H., personal communication). However, these data indicate that naltrexone is not an effective provocative test of hypothalamic-pituitary function in follicular phase female rhesus monkeys as it is in women. The possible contribution of pretreatment with a low dose of ketamine to these species differences cannot be evaluated from these data.

These findings on naltrexone are consistent with previous reports that the short-acting opioid antagonist, naloxone, is not effective in stimulating LH release during the early follicular phase in women or female rhesus monkeys (17,72). Naloxone is most effective in stimulating LH release during the late follicular and midluteal phase of the menstrual cycle (17,72). The differential effect of naloxone on LH during the early follicular and luteal phases of the menstrual cycle usually is attributed to differences in the ovarian steroid milieu as well as differences in LH secretory activity (17, 54, 67, 72). Relatively low rates of LH pulses and high levels of progesterone during the midluteal phase appear to facilitate the LH response to naloxone, whereas relatively high rates of LH secretory activity and low levels of ovarian steroid hormones during the early follicular phase are not conducive to naloxone stimulation of gonadotropins (54,67).

In an effort to evaluate the effect of ovarian steroid hormone levels on gonadotropin response to naltrexone, normal rhesus females in the late follicular phase of the menstrual cycle were studied also. But despite higher basal levels of E_2 and LH, there was no significant effect of naltrexone on LH. Naltrexone significantly suppressed E_2 and FSH in both the early and late follicular females (Figs. 1, 3 and 5). In human females, naltrexone significantly increased E_2 within 20 minutes, and FSH was not measured (63). Previous reports of naloxone's effects on FSH are inconsistent. Clinical studies in normal women have reported no FSH stimulation (57) and significant stimulation of FSH (61) at the same dose of naloxone (1.6 mg/hr). Naloxone (10 mg) significantly stimulated FSH within 45 min in the female chimpanzee during the early follicular phase (23). FSH was not measured in most previous studies of the effects of naloxone in female rhesus monkeys (54, 66, 67), but we observed no naloxone-induced changes in FSH in midluteal rhesus females (37).

Naltrexone-induced suppression of FSH without a parallel suppression of LH also supports the hypothesis that LH and FSH are regulated by different mechanisms (32). There is an extensive literature on inhibitory regulation of FSH (10). Inhibin, a nonsteroidal ovarian peptide, inhibits FSH release without affecting LH in several species (9, 10, 31) including man (33). Since inhibin was not measured in the present study, the basis for naltrexone's suppression of female rhesus FSH is unclear.

Naltrexone Effects on LH, FSH and T in Males

Naltrexone stimulated a significant increase in LH within 40 min and in T within 60 min at all doses studied (Fig. 2). The time course and magnitude of the naltrexone-stimulated increase in LH and T was similar to that previously observed after naloxone (0.50 mg/kg IV) administration (36). The peak LH response to naloxone or naltrexone stimulation occurred within 60 min in male rhesus monkeys (36), whereas the peak LH response to synthetic LHRH stimulation occurred within 30 min (64). These data are consistent with the hypothesis that opioid antagonists (naltrexone, naloxone) presumably act at the hypothalamus to stimulate release of endogenous LHRH and subsequent release of pituitary gonadotropins.

An increase in LH and T following naltrexone administration has been reported previously in rhesus and talapoin male monkeys (1,34). However, comparisons are limited by the fact that in those studies, samples were collected only once at 60 min postnaltrexone (1.0 mg/kg/IM) (1) or twice weekly during chronic naltrexone administration (0.50 mg/kg/IM, b.i.d.) (34). Our findings in male rhesus monkeys are consistent with previous reports that naltrexone (50 mg/PO) significantly increased plasma LH levels in normal and in opiate dependent men (40,45). FSH was unchanged after naltrexone administration in male monkeys (Fig. 3) and these findings are consistent with one previous report (1).

Naltrexone Effects on Prolactin

Prolactin was suppressed consistently following naltrexone administration in males and early follicular females (Fig. 4). These data are consistent with previous reports of naloxone-induced suppression of PRL in rhesus males (19,21). Most studies of naloxone's effects in rhesus females have not measured PRL (54, 66, 67), but we found that naloxone also suppresses PRL in rhesus females during the midluteal phase of the menstrual cycle (37). In contrast to these observations in monkey, opioid antagonists usually stimulate PRL in human females (8,41). Since opioid agonist drugs stimulate PRL both in humans and in monkey (19,72), this species difference in opioid antagonist effects on PRL is unexplained.

Naltrexone suppression of PRL cannot be attributed to unusual baseline levels of prolactin. Baseline PRL levels in males (20–26 ng/ml) were similar to those reported by Gold and co-workers (21) (21.3 ± 3.0 ng/ml). Baseline PRL levels in females (18–27 ng/ml) were similar to single venipuncture levels in females in this laboratory (20 ng/ml) and below levels measured in outdoor-housed females after novel capture (35 ng/ml) (3). However, differences in assay procedures between laboratories limit exact PRL level comparisons (2). Factors accounting for the inconsistent PRL response to naltrexone in late follicular females are unknown.

Gender Differences in Naltrexone Effects on the Hypothalamic-Pituitary-Gonadal Axis

Naltrexone's opposite effects on LH, FSH and gonadal steroid hormones in male and female rhesus monkeys are probably due to biological rather than methodological variables. The same dose of naltrexone was used in males and females under identical conditions, so it is impossible to explain this apparent gender difference in terms of procedural differences. Another possible contributing factor is that males had relatively higher basal levels of gonadal steroids than early follicular females. But higher gonadal steroid levels in late follicular females did not increase the LH response to naltrexone. Average E_2 levels in these late follicular females were consistent with the early ascending limb of the peri-ovulatory E_2 surge (29).

There is a possibility that gender-related differences in the hypothalamic-pituitary-adrenal axis may contribute to these findings. It is known that the hypothalamic-pituitary-adrenal

axis modulates hypothalamic-pituitary-gonadal secretory activity and that naltrexone stimulates ACTH in females (42, 53, 63). However, no data on naltrexone effects on ACTH are currently available in males. It is tempting to speculate that naltrexone-stimulated increases in corticotropin-releasing factor (CRF) may have contributed to the absence of LH stimulation in females and to suppression of E_2 and FSH. FSH is not affected by administration of CRF in rat (60), but exogenous CRF inhibits both LH and FSH in ovariectomized rhesus females (53). Other than a direct effect of naltrexone on ACTH, it is difficult to argue that some nonspecific "stress" associated with the experimental situation had a greater impact on females than males.

These data suggest that there are gender differences in the endogenous opioid regulation of pituitary gonadotropin release in adult rhesus monkeys. This conclusion is consistent with developmental studies in rats which demonstrate gender and age specific differences in sensitivity to naloxone which persist past puberty into early adulthood (14). Similarly, striking gender differences in LH and FSH secretory activity were observed in infant rhesus males and females, castrated at one week of age (56). These data in infant rhesus castrates were interpreted to suggest a fundamental gender difference in hypothalamic LHRH pulse frequency which is independent of the prevailing gonadal steroid milieu (56). The mechanisms underlying the gender differences in the hypothalamic-pituitary response to naltrexone observed in the present study remain to be determined.

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