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Acute Effects of Nalmefene on LH, Prolactin, and Testosterone in Male Rhesus Monkeys

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MELLO, N. K., J. H. MENDELSON AND M. KELLY. *Acute effects of nalmefene on LH, prolactin, and testosterone in male rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **66**(2) 275–284, 2000.—The effects of the long-acting opioid antagonist, nalmefene [17-N-cyclopropylmethyl-3,14-β-dihydroxy-4,5-α-epoxy-6-methylene morphinan hydrochloride] on LH, T, and prolactin release in rhesus monkeys are unknown. The acute effects of nalmefene (0.01 and 0.10 mg/kg, IV) or placebo on LH, PRL, and T were studied, and samples were collected at 10-min intervals for 360 min to permit cluster analysis of pulsatile release patterns. LH increased significantly within 30 min after nalmefene, and remained significantly above baseline levels for 50 to 60 min ($p < 0.05$). Testosterone increased significantly within 70 to 80 min after nalmefene, and remained significantly above baseline for 60 min ($p < 0.05$). Although nalmefene antagonizes opioid agonists for 6–8 h, inhibitory feedback by testosterone appeared to limit the duration of its antagonism of endogenous opioid inhibition of LHRH and stimulation of LH. Nalmefene did not change LH or PRL pulse frequency or amplitude significantly in comparison to placebo administration. © 2000 Elsevier Science Inc.

Nalmefene Luteinizing hormone Prolactin Testosterone Opioidantagonist

NALMEFENE [17-N-cyclopropylmethyl-3,14-β-dihydroxy-4,5-α-epoxy-6-methylene morphinan hydrochloride] is an opioid receptor antagonist derived from naltrexone (19). Both clinical and preclinical studies have shown that nalmefene has a longer duration of opioid antagonist action than either naltrexone or naloxone (9,12,13,15,38), and the Food and Drug Administration has approved nalmefene (REVEX®) for treatment of opioid overdose (33). Nalmefene effectively reverses opioid agonist-induced respiratory depression for 8 h or more, and the duration of nalmefene's antagonist effects were dose related over a range of 0.5 to 2 mg (13). In contrast, a short-acting opioid antagonist, such as naloxone, may be effective for about 2 h (15,45). In rhesus monkeys, nalmefene (0.01 mg/kg) antagonized the behavioral effects of morphine for more than 6 h, whereas the same dose of naltrexone was effective for less than 4 h (12). In ovariectomized rhesus monkeys, nalmefene (10 mg, IV) blocked a morphine-induced (10 mg, IV) inhibition of luteinizing hormone (LH) and stimulation of prolactin for at least 24 h (38).

Another potential therapeutic application of long-acting opioid antagonists is for the treatment of hypothalamic amenorrhea [(26,47); see (27) for review]. It is well established that the pulsatile release of LH from pituitary gonadotropes is stimulated by hypothalamic luteinizing-hormone releasing-hormone (LHRH), and LHRH release is under the inhibitory control of endogenous opioid peptides (7,20,43,51). Opioid antagonists stimulate gonadotropin release, presumably by antagonizing the inhibitory effects of endogenous opioids on LHRH (7,51). Studies of the effectiveness of naltrexone in treating hypothalamic amenorrhea, or amenorrhea secondary to hyperprolactinemia, have been inconsistent, and both positive (1,21,47,48) and negative findings have been reported (6,8). Clinical evaluations of nalmefene are consistent with the hypothesis that this opioid antagonist stimulates LH release. Nalmefene (20 mg, PO) increased the amplitude but not the frequency of LH pulses in female athletes with oligo-amenorrhea (22). In normal men, nalmefene (10 mg, PO) increased the frequency of LH pulses, but not LH pulse amplitude (17). Nalmefene (2.0 mg, IV, t.i.d.) also significantly increased basal levels of LH and testosterone in elderly men with impotence, but pulsatile release characteristics of these hormones were not measured (4). In normally cycling rhesus females, nalmefene (10 mg, b.i.d.) enhanced an estrogen-induced LH surge and these effects were inhibited by progesterone (37).

Although opioid agonists usually stimulate prolactin release (7,14,16,38,50), opioid antagonists have inconsistent ef-

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fects on basal prolactin levels. For example, naltrexone (0.50 to 1.0 mg/kg, IV) significantly decreased prolactin levels in both male and female rhesus monkeys (24), but naltrexone (50 mg, PO) had no effect on prolactin levels in human males with a history of heroin addiction (10). Nalmefene (0.5 or 5.0 mg, IV) stimulated an increase in prolactin in normal rhesus females whereas naloxone (0.5 and 5.0 mg, IV) did not (38). Corticotropin-releasing-hormone (CRH) stimulated a dose-dependent increase in prolactin release in both intact and ovariectomized female monkeys. This CRH-induced prolactin release was inhibited by pretreatment with naloxone, but not by pretreatment with nalmefene (39). It was suggested that these differences between naloxone and nalmefene on CRH-stimulated prolactin release might reflect the kappa opioid activity of nalmefene (39). However, the implications of kappa opioid activity for endogenous opioid regulation of prolactin are unclear. Nalmefene has affinity for mu, kappa, and delta opioid receptors in both rat and monkey brain membranes (11,29). Although nalmefene had a greater affinity for kappa and delta receptors than either naloxone or naltrexone in binding studies in rat brain membranes (29), this does not appear to be the case in rhesus monkeys. In rhesus monkey brain membranes, the binding affinity of nalmefene and naltrexone was similar for mu, kappa, and delta receptors, and selectivity for kappa/mu receptors was also similar (11). Behavioral studies in rhesus monkeys found that although nalmefene antagonized the antinociceptive effects of both mu (alfentanil) and kappa (enadoline) receptor opioid agonists, it has greater selectivity for mu than kappa receptors (12). These data led to the conclusion that nalmefene is qualitatively similar to naltrexone in rhesus monkeys (12).

One goal of the present study was to examine the magnitude and duration of the effects of single doses of nalmefene on LH and prolactin in male rhesus monkeys. Males were selected for study so that hormonal changes associated with phases of the menstrual cycle could not contribute to pituitary gondotroph sensitivity [cf, 44]. In view of the long duration of opioid antagonist activity after exogenous opioid administration, we were also interested to learn the duration of nalmefene's antagonism of endogenous opioid inhibition of LH release.

There is considerable evidence that gonadal steroid feedback regulation of LH involves endogenous opioid systems (40). A second goal of this study was to examine the interactions between gonadal steroid feedback and nalmefene's stimulatory effects on LH. The effects of nalmefene on interactions between LH and testosterone have not been studied previously in male rhesus monkeys. Stimulation of LH is usually followed by increased testosterone levels, which in turn inhibit LH release (2). In previous studies, the opioid antagonist naltrexone stimulated LH release, and this was followed by increases in testosterone in male rhesus monkeys (24). A third objective was to quantify LH and prolactin pulsatile release patterns using Cluster analysis procedures developed by Veldhuis and Johnson (41), and to compare the effects of placebo and nalmefene on patterns of LH and prolactin pulsatile release under identical experimental conditions in the same subjects.

METHOD

Subjects

Five adult male rhesus monkeys (*Macaca mulatta*) (7.6 to 11.4 kg) lived in individual cages and were maintained on ad lib food and water. Lab Diet Jumbo Monkey Biscuits (PMI Foods, Inc., St. Louis, MO) were supplemented with fresh fruit, vegetables, and multiple vitamins each day. Monkeys were fed twice each day at 0900 and 1700 h. A 12 hr light-dark cycle (0700–1900 h) was in effect. These monkeys were experimentally naive and did not have a chronic drug exposure history. Each monkey was adapted to placement in a standard primate restraining chair on several occasions before these studies were initiated. Each monkey was studied as its own control on three occasions, after placebo and low and high dose nalmefene administration. Successive studies were separated by at least 2 months.

Animal maintenance and research were conducted in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, NIH. This protocol was approved by the Institutional Animal Care and Use Committee. The facility is licensed by the U.S. Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian trained in primate medicine.

Nalmefene and Placebo Administration

The acute effects of saline-placebo and a low (0.01 mg/kg, IV) and a high (0.1 mg/kg, IV) dose of nalmefene on basal levels of LH, PRL, and T were evaluated. Saline-placebo or nalmefene were administered as an IV bolus over 1 min. Treatments were given in an irregular order, counterbalanced across subjects. These doses of nalmefene were selected on the basis of previous studies conducted in rhesus monkeys (12). The low dose of nalmefene antagonized morphine's antinociceptive effects for over 6 h in rhesus monkeys (12), and we hypothesized that this dose should be sufficient to stimulate LH release. A 10-fold higher dose also was studied to determine if the magnitude or duration of nalmefene's effects on anterior pituitary and gonadal hormones were dose dependent over this range. In clinical studies that used similar endocrine end points, nalmefene was administered to men at doses of 2.0 mg, t.i.d. (which is equivalent to 0.11 mg/kg in a 70 kg man) (4), 10 mg, p.o. (which is equivalent to 0.14 mg/kg in a 70 kg man) (17), and to women at doses of 20 mg, p.o. (which is equivalent to 0.36 mg/kg in a 55 kg woman) (22). Nalmefene at these doses increased basal levels of LH in both men and women (17,22) and T in men (4).

Acute Venous Catheter Implantation and Blood Sample Collection

Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg, IM). A Sur-Flo Intercath containing a 20 gauge needle (i.d. 0.80 × 51 mm, Terumo Medical Corporation, Elkton, MD) was inserted into the saphenous vein using aseptic techniques. After removal of the needle stylet, the catheter was joined to heparin-impregnated sterile silicon tubing and secured with sutures. A second catheter for intravenous infusion of saline control or nalmefene solutions was implanted in the opposite leg. After drug or saline infusion, a 0.9% NaCl solution was infused at a rate of 20 ml/h. Each monkey was placed in a standard monkey chair for 2 h before sample collection began to reduce any possible stress associated with the catheter implantation procedure and to ensure that any effects of the ketamine had dissipated. Food treats were offered after the first hour to minimize any possible effects of short-term food restriction on LH pulsatile release [see (5) for review]. Blood samples for LH, PRL, and T analysis were collected in heparinized tubes. Samples were centrifuged, and aliquots of plasma were drawn and stored at −70°C until analysis.

Basal levels of LH, PRL, and T were measured 10 min before placebo or nalmefene was administered. Following IV

nalmefene or placebo administration, samples for analysis of LH, PRL, and T were collected at 10-min intervals for 360 min. This frequency and duration of sampling was used to permit quantitative characterization of the frequency and amplitude of LH and prolactin pulsatile release profiles using the computer algorithms developed by Veldhuis and Johnson (41). It is well established that LH and PRL are released in a pulsatile manner from gonadotropes and lactotropes in the anterior pituitary (42,50), and the sample collection procedures used in this study were similar to those used to study pulsatile release patterns of LH and prolactin in clinical studies (28,42). Although there has been considerable debate about the feasibility of measuring LH pulsatile release in chair-restrained rhesus monkeys, it has been determined that after repeated chair adaptation, identical LH release patterns were measured in chair-restrained rhesus females and in tether-restrained rhesus females using a remote sampling system (32).

Drug Preparation

Nalmefene hydrochloride was purchased from the Sigma Chemical Co., St. Louis, MO, and solutions were prepared by dissolving nalmefene in sterile water for injection U.S.P. The solution was filter-sterilized using a 0.22 micron Millipore Filter (Bedford, MA).

Plasma Hormone Analyses

Data are reported for the analysis of LH, PRL, and T determinations. Details of the assay procedures are as follows.

LH radioimmunoassay. Plasma LH concentrations were determined in duplicate by a double-antibody radioimmunoassay procedure similar to that described by Midgley (30), using materials prepared by Dr. W. Peckham and following his suggestions. Purified ceropithecus pituitary LH for radioiodination (WP-XV-117-3239), rabbit antiserum (WP-R13, pool D) to human choriogonadotropin, 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 performed using chloramine-T (18) with sodium iodide-125 purchased from New England Nuclear Life Science Products (Billerica, MA). Goat antirabbit gammaglobulin was obtained from Cal Biochem-Nova Biochem Corp. (La Jolla, CA). Results are expressed in nanograms per milliliter in terms of the reference preparation. The LH assay sensitivity was 7.2 ng/ml. Intra- and interassay CVs were 7.7 and 8.4%, respectively.

PRL radioimmunoassay. Plasma prolactin concentrations were measured using a double antibody radioimmunoassay kit (Pantex, Santa Monica, CA). Results are expressed in nanograms per milliliter in terms of the reference preparation. The PRL assay sensitivity was 1.8 ng/ml. Intra- and interassay CVs were 8.7 and 13.0%, respectively.

Testosterone radioimmunoassay. Plasma testosterone concentrations were measured using a direct, double-antibody radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA). The assay sensitivity was 2.8 ng/dl. Intra- and interassay CVs were 11.2 and 11.9%, respectively.

Statistical Analysis

The effects of nalmefene and placebo on LH (ng/ml), prolactin (ng/ml) and testosterone (ng/dL) were evaluated with analysis of variance (ANOVA) for repeated measures (Super ANOVA, Abacus Concepts, Inc., Berkeley, CA, 1989). ANOVA for repeated measures was used to compare group mean values at each sample period with baseline means using contrast tests. If ANOVA showed a significant main effect, contrast tests were used to determine which points were statistically different from each other. Probability levels of $p < 0.05$ or above are reported as statistically significant. Group data are displayed as percent change from baseline to facilitate comparisons between LH and T.

Discrete Endocrine Peak Detection and Analysis

The Cluster analysis program, which serially scans endocrine data series for "clusters" of significantly increased or decreased values (41) was used to identify LH and prolactin pulses and to quantify pulse frequency and amplitude. This algorithm has been validated for false positive errors on signal-free noise (36). We used a conservative three-point test nadir

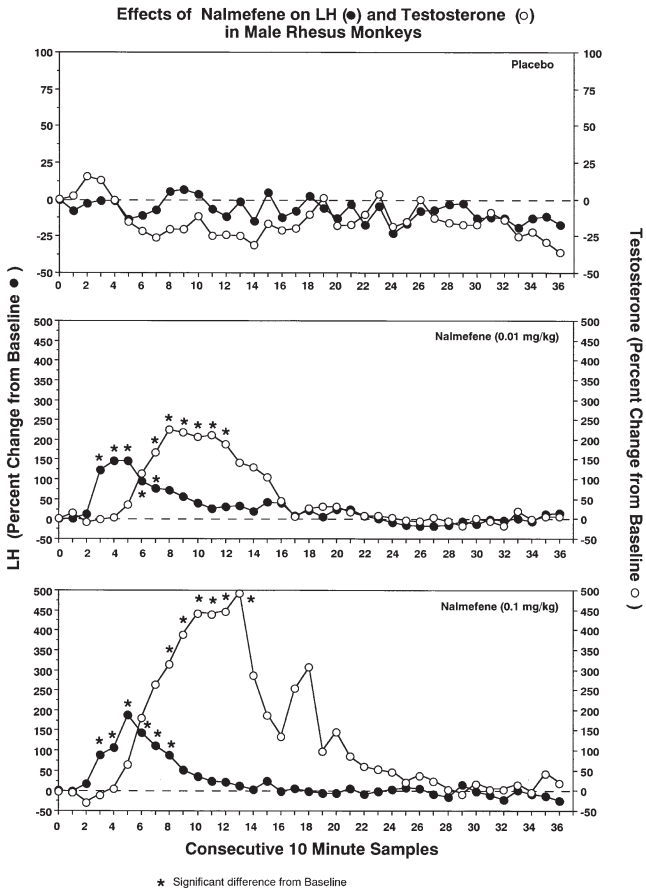

FIG. 1. The effects of placebo and nalmefene on LH and testosterone. The abscissae show consecutive samples collected at 10-min intervals after IV placebo and nalmefene (0.01 and 0.10 mg/kg, IV) administration. The left ordinate shows LH levels (ng/ml) expressed as percent change from baseline (closed circles) during each of the three conditions. The right ordinate shows testosterone levels (ng/dl) expressed as percent change from baseline (open circles). Each data point is based on the average (±SEM) LH or T level in four or five monkeys. Statistically significant changes from the preplacebo or prenalmefene baseline are indicated by asterisks ($p < 0.05$).

and a two-point test peak and a statistic of 3/3 for significant upstrokes and downstrokes to identify peaks. Experimental measurement variance inherent in the sample replicates was modeled as a power function of hormone concentration (35). Various pulse parameters, including number of peaks and peaks per hour, interpulse intervals (min), peak amplitude (maximal peak height), fractional peak amplitude (percentage increase over preceding nadir), and interpulse mean valley concentration were estimated in each of the time series. Each of the pulse parameters measured after nalmefene administration was compared with its respective placebo control baseline with ANOVA for repeated measures.

RESULTS

Baseline Levels of LH, Prolactin, and Testosterone

There were no statistically significant differences in baseline LH, PRL, or T levels (mean ± SE) across placebo and nalmefene administration conditions. Baseline levels of LH averaged 19.9 (±1.2), 14.6 (±2.6), and 17.2 (±0.9) ng/ml before administration of placebo and low and high doses of nalmefene, respectively. Baseline levels of PRL averaged 13.5 (±4.6), 12.0 (±4.6), and 12.5 (±4.9) ng/ml before administration of placebo and low and high doses of nalmefene, respectively. Baseline levels of T averaged 133.7 (±36), 190.4 (±77), and 184.5 (±45) ng/dl before administration of placebo and low and high doses of nalmefene, respectively.

LH and Testosterone Levels After Nalmefene and Placebo Administration (Group Data)

The effects of placebo and each dose of nalmefene on basal levels of LH and T over 360 min are shown in Fig. 1. Placebo administration had no significant effects on LH or T. In contrast, each dose of nalmefene stimulated a significant increase in LH from baseline within 30 min ($p < 0.05$) and LH reached peak levels within 50 min. After the low dose of nalmefene, LH increased 147% above baseline and averaged 36 (±9.5) ng/ml. After the high dose of nalmefene, LH increased to 187% above baseline and averaged 50 (±12.6) ng/ml. Although peak LH increases were greater after the higher dose of nalmefene, these differences between peak LH levels after low and high doses of nalmefene were not statistically significant. LH remained significantly above baseline for 50 min after 0.01 mg/kg nalmefene and for 60 min after 0.10 mg/kg nalmefene. LH levels did not differ significantly from prenalmefene baseline levels within 80 min after low dose nalmefene administration and within 90 min after high dose nalmefene administration.

Testosterone also increased significantly within 70 to 80 min after nalmefene administration ($p < 0.05$). Peak levels of T were measured within 80 to 130 min after nalmefene. After the low dose of nalmefene, T increased over 200% above baseline and averaged 618 (±182) ng/dl at peak. After the high dose of nalmefene, T increased to 500% above baseline and averaged 1004 (±444) ng/dl at peak. Testosterone remained significantly above baseline for 60 min after both the low and high dose of nalmefene, then gradually decreased. Testosterone did not differ significantly from prenalmefene baseline levels within 130 min after low dose nalmefene administration and within 140 min after high dose nalmefene administration.

Peak levels of LH coincided with the initial increase in testosterone after both doses of nalmefene. Significant increases in testosterone were detected within 40 to 50 min after significant increases in LH. LH levels began to decrease as testosterone levels rose and LH did not differ significantly from baseline within 20 to 30 min after testosterone levels increased significantly above baseline.

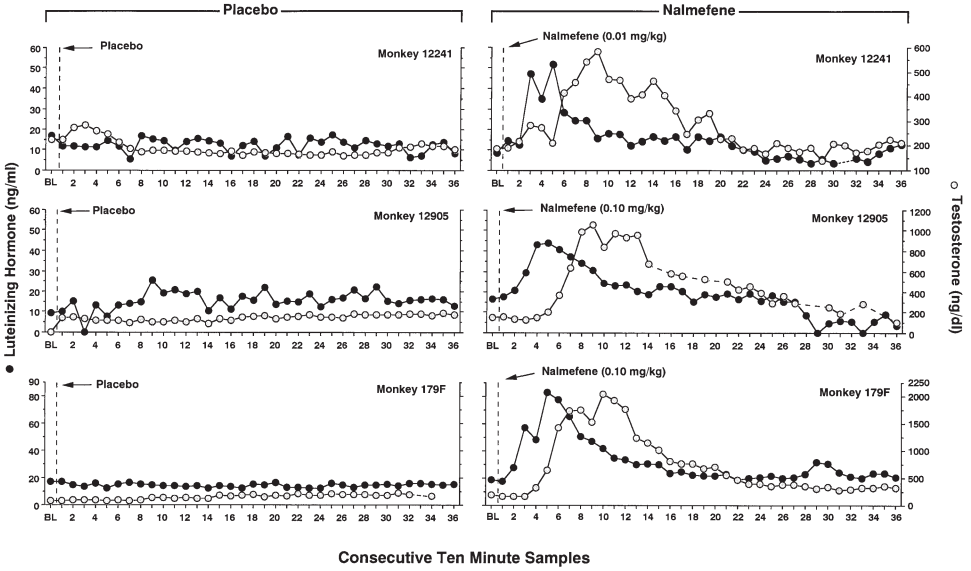

FIG. 2. The effects of placebo and nalmefene on LH and testosterone in individual male rhesus monkeys. The abscissae show consecutive samples collected at 10-min intervals before (BL) and after placebo (left column) and nalmefene (right column) administration at the vertical dotted line. The left ordinate shows LH levels (ng/ml) (closed circles), and the right ordinate shows testosterone levels (ng/dL) (open circles).

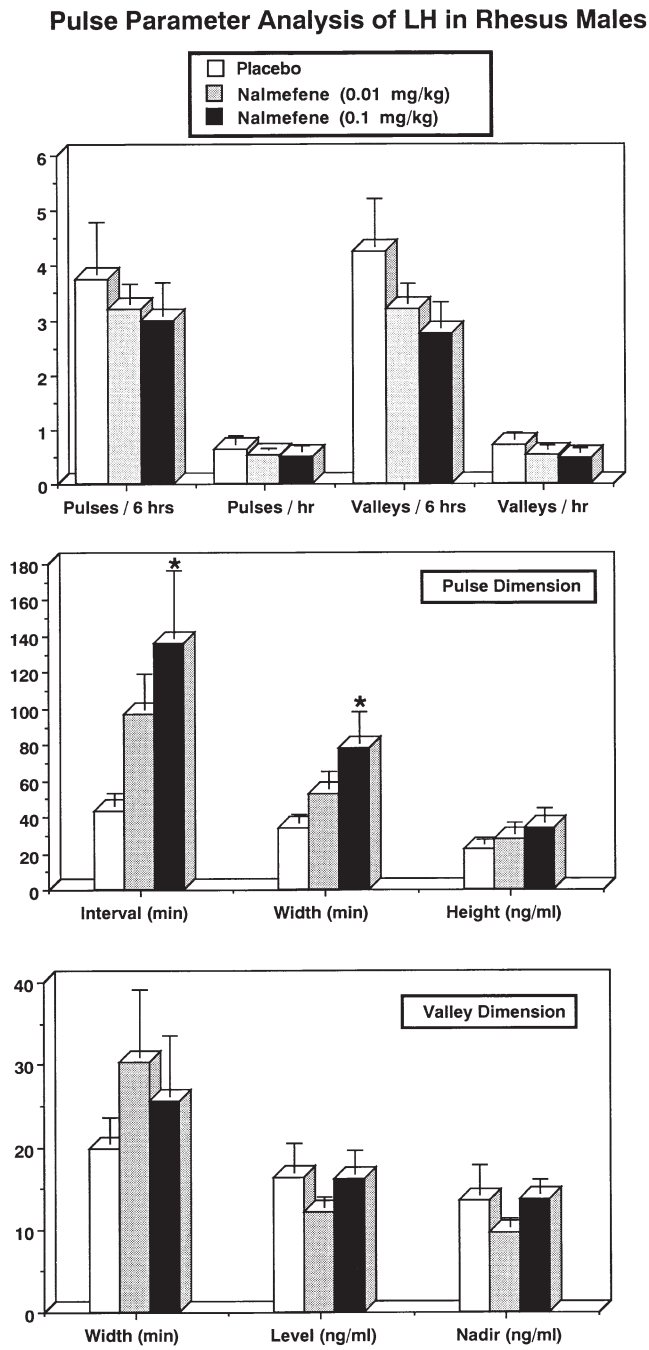

FIG. 3. Patterns of LH pulsatile release after placebo and nalmefene. Pulsatile release patterns of LH were calculated for each individual monkey by the Cluster analysis program (41) and group data (average ± SEM) for the number of pulses and valleys, and for pulse and valley dimensions after placebo and nalmefene administration are shown in each row. Placebo conditions are shown as an open rectangle; nalmefene (0.01 mg/kg, IV) is shown as light gray rectangles, and nalmefene (0.10 mg/kg, IV) is shown as a black rectangle for each variable. In row 1, the number of pulses detected over the 360-min sampling period, the number of pulses per hour, the number of valleys and valleys per hour are shown. In row 2, the pulse dimensions (interpulse interval and pulse width in minutes) and pulse height (ng/ml) are shown. In row 3, the valley dimensions, valley width, valley level, and nadir are shown. Statistically significant changes from baseline are indicated by asterisks ($p < 0.05$).

LH and Testosterone Levels After Nalmefene and Placebo Administration (Individual Data)

The effects of placebo and one dose of nalmefene on basal levels of LH and T are shown for three individual monkeys in Fig. 2. Placebo administration did not change LH and T across the 360-min sampling period. After nalmefene administration, peak levels of LH were measured within 50 min and peak levels of T were measured within 90 min (Fig. 2, rows 1 and 2) or 100 min (Fig. 2, row 3). In each instance, LH levels began to decrease as T levels began to rise. There was considerable individual variability in the magnitude of the increase in T.

Pulsatile Release of LH After Nalmefene and Placebo Administration

Figure 3 shows the effects of nalmefene and placebo on the pulsatile release of LH as determined by Cluster analysis (41). Cluster analysis of pulse and valley frequency and quantitative characteristics of the peak and valley dimensions are based on group data. After placebo administration, an average of 3.75 (±0.9) LH peaks were detected over 6 h (mean ± SE) (row 1). Nalmefene administration did not change LH pulse frequency significantly. After low dose nalmefene administration, LH pulse frequency averaged 3.20 (±0.37) peaks per 6 h (mean ± SE). After high dose nalmefene administration, LH pulse frequency averaged 3.0 (±0.58) peaks per 6 h (mean ± SE). Analysis of the LH pulse dimensions is shown in Fig. 3, row 2. The LH interpulse interval and pulse width increased with increasing doses of nalmefene but these differed significantly from placebo only at the highest dose of nalmefene ($p < 0.05$). After the initial surge in LH, the amplitude of LH pulses detected did not differ across conditions. Analysis of LH valley dimensions is shown in Fig. 3, row 3. Although there were variations in valley width, level and nadir, these were not consistently nalmefene dose related, and did not differ significantly from placebo.

Prolactin Levels After Nalmefene Administration

The effects of placebo and each dose of nalmefene on prolactin levels over 360 min are shown in Fig. 4. There were no significant changes in prolactin from baseline levels after placebo or nalmefene administration. Although placebo administration had no statistically significant effects on PRL, there was considerable variability in PRL during the first 2 h of sample collection after placebo administration, then PRL levels stabilized to average between 7.3 and 8.7 ng/ml throughout the remaining 4 h of the sampling period. After high-dose nalmefene administration, prolactin levels increased slightly from baseline, but this change was not statistically significant.

Pulsatile Release of Prolactin After Nalmefene and Placebo Administration

Figure 5 shows the effects of nalmefene and placebo on the pulsatile release of PRL as determined by Cluster analysis (41). There were no consistent nalmefene dose-related effects on any PRL peak or valley dimension measured by the Cluster analysis computer algorithm. The total number of prolactin peaks detected after placebo administration averaged 3.5 (±0.29) per 6 h. After low- and high-dose nalmefene administration, an average of 4.0 (±0.41) and 3.25 (±0.85) peaks (mean ± SE) were detected during the 360-min sampling period. The average number of peaks per hour detected over the 6-h sampling period averaged 0.58 (±0.05), 0.67 (±0.07), and

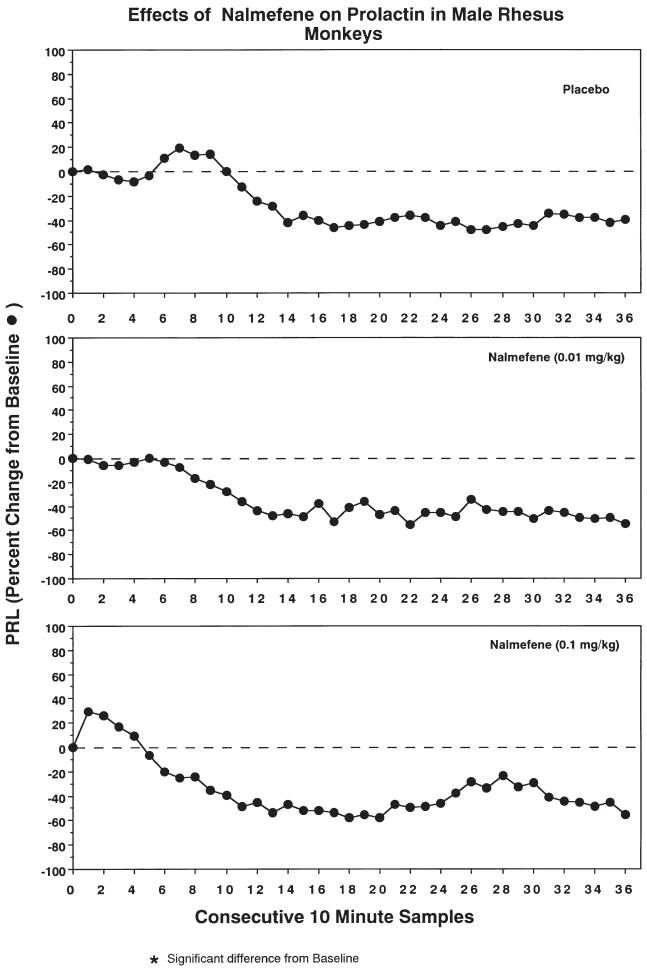

FIG. 4. The effects of placebo and nalmefene on PRL. The abscissae show consecutive samples collected at 10-min intervals after IV placebo and nalmefene (0.01 and 0.10 mg/kg, IV) administration. The left ordinate shows PRL levels (ng/ml) expressed as percent change from baseline during each of the three conditions. Each data point is based on the average (±SEM) PRL level in four or five monkeys.

0.54 (±0.14) (mean ± SE) after placebo and low- and high-dose nalmefene, respectively.

DISCUSSION

One major finding of this study was that the opioid antagonist, nalmefene, stimulated a significant increase in LH that was followed by a significant increase in testosterone. However, the duration of nalmefene's stimulation of LH was relatively brief in comparison to nalmefene's antagonism of the sedative, respiratory, and antinociceptive effects of opioid agonists (12,13,15). Some implications of these findings in relation to the current literature on opioid antagonist interactions with anterior pituitary and gonadal hormones are discussed below. Nalmefene had no significant effect on prolactin in male rhesus monkeys studied under these conditions, and these data are consistent with previous studies of the parent compound, naltrexone, in human males (10). Another major finding was that pulsatile release patterns of LH and prolactin could be measured in chaired rhesus monkeys, and this is in agreement

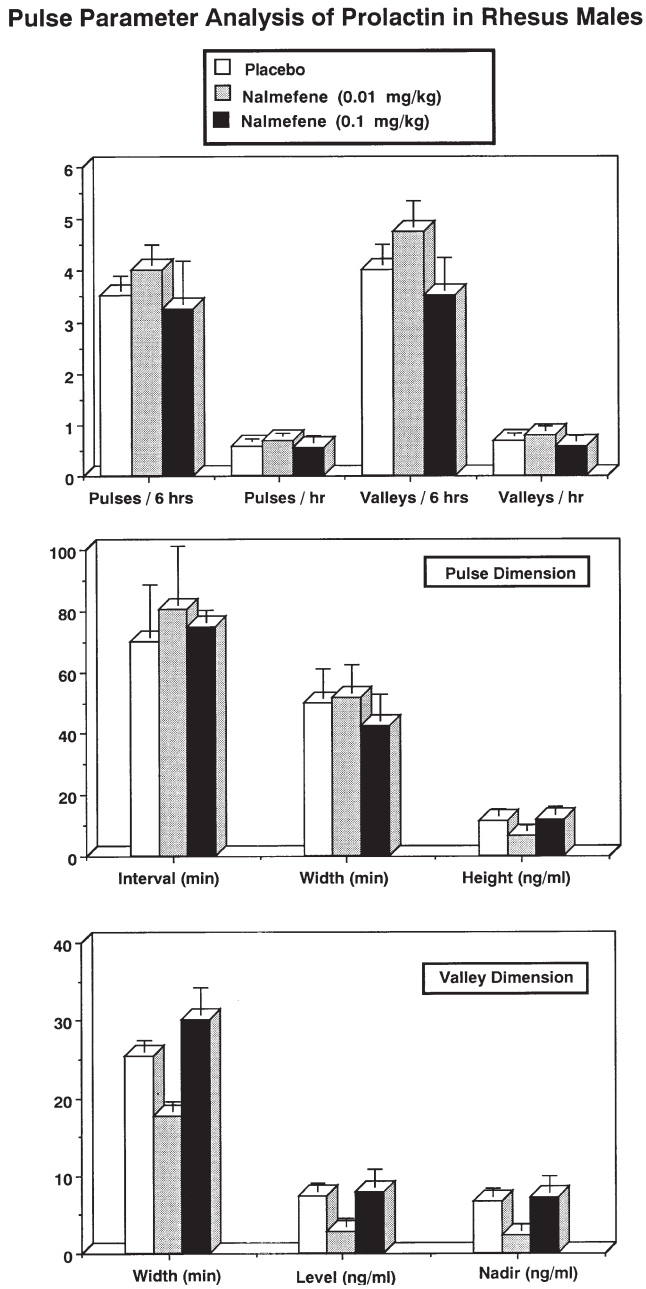

FIG. 5. Patterns of PRL pulsatile release after placebo and nalmefene. Pulsatile release patterns of PRL were calculated for individual monkeys by the Cluster analysis program (41) and group data (average ± SEM) for the number of pulses and valleys, and for pulse and valley dimensions after placebo and nalmefene administration are shown in each row. Placebo conditions are shown as an open rectangle; nalmefene (0.01 mg/kg, IV) is shown as light gray rectangles, and nalmefene (0.10 mg/kg, IV) is shown as black rectangles for each variable. In row 1, the number of pulses detected over the 360-min sampling period, the number of pulses per hour, the number of valleys and valleys per hour are shown. In row 2, the pulse dimensions (interpulse interval and pulse width in minutes) and pulse height (ng/ml) are shown. In row 3, the valley dimensions, valley width, valley level, and nadir are shown. Statistically significant changes from baseline are indicated by asterisks ($p < 0.05$).

with previous reports (32). Moreover, under placebo control conditions, PRL release patterns in these male rhesus monkeys were identical to those measured in human males studied with similar sample collection and analytic procedures (28).

Basal Levels of Luteinizing Hormone and Testosterone After Placebo Administration

Basal levels of LH and T were consistent with levels previously measured in male rhesus monkeys in this laboratory [(24,25); see (23) for review]. Most studies of the pulsatile release of LH in rhesus monkeys have been conducted in ovariectomized females or in females at various phases of the menstrual cycle (31,32). The LH pulsatile release patterns detected in the present study with the Cluster analysis program (3.75 pulses/6 h) were more frequent than reported in a previous study of male rhesus monkeys analyzed with the pulsar program (4.57 pulses/12 h) (5). Although differences in analytic and statistical procedures limit detailed comparisons between these studies, LH pulsatile release patterns obtained in the present study appear to be within the normal range for rhesus monkeys. In normal rhesus females where LH samples were collected at 10-min intervals over 4 or 8 h and analyzed with the Cluster analysis program an average of one LH pulse per hour was detected during the follicular phase and one LH pulse per 4 h was detected during the luteal phase (32). Earlier studies reported 14 to 15 LH pulses every 12 h in follicular phase rhesus females (31). Administration of synthetic LHRH at the rate of one pulse per hour was effective in restoring normal patterns of gonadotropin release in ovariectomized monkeys with lesions of the arcuate nucleus and the median eminence (20). Importantly, data obtained in the present study confirm previous reports that pulsatile release of LH can be measured in chaired rhesus monkeys, if monkeys are well adapted to chair restraint (32).

LH and Testosterone Interactions with Nalmefene

The significant increase in LH after both low- and high-dose nalmefene administration was expected on the basis of an extensive literature showing that regulation of hypothalamic LHRH release is under inhibitory control by endogenous opioid peptides (7,20,43,51). LH increased significantly above baseline levels within 30 min after low- and high-dose nalmefene administration, and reached peak levels within 50 min. These data are consistent with an earlier report that the parent compound, naltrexone (0.25, 0.50, and 1.0 mg/kg, IV) increased LH significantly to 109, 158, and 167% above baseline within 40 min in male rhesus monkeys (24). The duration of nalmefene stimulation of LH (50 to 60 min) was similar to that observed after naltrexone stimulation of LH (40 to 60 min) in male rhesus monkeys (24).

However, the relatively short duration of nalmefene's effects on LH was surprising in view of its long duration of action on behavioral and endocrine end points under conditions where exogenous opioids were administered. For example, in ovariectomized rhesus monkeys, nalmefene (10 mg, IV), given 12 or 24 h before morphine (10 mg, IV) administration, blocked morphine's inhibition of LH and stimulation of PRL (38). Nalmefene (0.01 mg/kg, IV) also blocked the antinociceptive effects of exogenous opioid agonists for over 6 h in rhesus monkeys (12). In clinical studies, nalmefene (2 mg, IV) antagonized opioid agonist effects for 8 h or more (9). Insofar as LH stimulation reflects nalmefene's antagonism of endogenous opioid inhibition of LHRH, it might be anticipated that this long-acting opioid antagonist would produce sustained increases in LH. However, negative feedback inhibition by testosterone and/or limitations on LH synthesis and release may have influenced the duration of LH stimulation.

Reciprocal control of LH and testosterone through negative feedback mechanisms has been well established (2). This was illustrated in the present study by the significant increase in T within 40 to 50 min after nalmefene stimulation of significant increases in LH. Subsequent negative feedback inhibition of LH by increasing levels of T is the most likely explanation for the relatively short duration of LH stimulation after nalmefene administration. We observed similar interactions between LH and T after administration of the opioid antagonist naltrexone (24) and after administration of a high dose of cocaine (25). Naltrexone and cocaine each stimulated LH within 20 to 40 min, and this was followed by a significant increase in testosterone (24,25). After both naltrexone and cocaine administration, the interval between peak LH levels and peak testosterone levels was about 60 min (24,25). Evidence that steroid feedback regulation of LH release involves endogenous opioid systems is illustrated by the finding that when LH pulsatile release was suppressed by continuous infusion of 5-α-dihydrotestosterone, concurrent administration of naltrexone (1.0 mg/kg, PO) restored LH pulse frequency to control levels (40).

Alternatively, it is also possible that the duration of nalmefene's stimulation of LH reflects limitations on the capacity of pituitary gonadotrophs to synthesize and release LH rather than limitations on the opioid antagonist effects of nalmefene. Changes in pituitary sensitivity to exogenous challenges and pituitary reserve as a function of menstrual cycle phase have been well documented (44). The limitations on and determinants of pituitary reserve in male rhesus monkeys are not well understood and the role of pituitary reserve in nalmefene's effects on LH is unknown. Taken together, these data indicate that the duration of opioid antagonist effects on hormones regulated by endogenous opioid systems cannot be predicted from opioid antagonist effects on exogenous opioid-induced sedation, respiratory depression or antinociception.

Nalmefene and LH pulsatile release. There was a nonsignificant decrease in LH pulse frequency after nalmefene in comparison to placebo and a corresponding significant increase in the interval between pulses as well as pulse width. It is likely that the rapid increase in LH stimulated by nalmefene, combined with the subsequent negative feedback inhibition of LH by testosterone, contributed to the pulsatile release patterns observed. We are unaware of any previous studies of nalmefene's effects on LH pulsatile release patterns in male rhesus monkeys. One clinical study examined the effects of oral nalmefene (20 mg) on LH release patterns in female athletes who had been oligo-amenorrheic for 1 to 7 years (3.5 years average) (22). Samples were collected every 10 min for 6 h before and 6 h after nalmefene or placebo administration and analyzed with the pulsar program (22). The frequency of LH pulses did not differ between conditions, but LH pulse amplitude was significantly greater after nalmefene administration than before nalmefene in women (22). These clinical data in women are not consistent with a report of nalmefene's effects on LH in normal men (17). In that study, LH pulse frequency, but not amplitude, increased significantly after oral nalmefene administration, and this was accompanied by a significant increase in testosterone. The time course of changes in LH and T after oral nalmefene were not reported (17).

Baseline levels of prolactin. Under control conditions, prolactin levels were in the normal range (13.5 ng/ml) for drug-

naive rhesus males studied in this laboratory [(25), see (23) for review]. Moreover, the prolactin pulsatile release patterns measured in this study after placebo administration were almost identical to previous studies of human males in several respects. In normal men, prolactin pulse frequency averaged 3.25 (±0.16) (mean ± SE) peaks per 6 h with an interpulse interval of 85.8 (±5.4) (mean ± SE) min when samples were collected at 10-min intervals over 6 h and analyzed with the Cluster analysis program (28). Under the conditions of the present study, 3.5 (±0.3) prolactin pulses were detected over 6 h, and the average interpulse interval was 70 (±16.8 min). The similarity of prolactin pulsatile release patterns in men and in rhesus monkeys is further evidence of the validity of this animal model for endocrine studies. Prolactin pulsatile release in these rhesus males and in human males (28) was more rapid than in intact rhesus females [3.9 (±0.6) pulses per 12 h] and ovariectomized rhesus females [2.3 (±0.2) pulses per 12 h] (46). Differences in sampling frequency (30 vs. 10 min), duration (12 vs. 6 h) and experimental conditions (pentobarbital vs. nalmefene administration) do not permit conclusions about whether or not these differences in prolactin release can be attributed to gender differences. Interestingly, a more rapid rate of PRL pulsatile release (one pulse every 8 to 10 min) was measured in isolated perifused hemipituitaries obtained from male and female *Macaca nemestrina* monkeys (34).

Prolactin Interactions with Nalmefene

Prolactin is generally agreed to be under inhibitory dopaminergic control and direct and indirect dopamine agonists usually decrease prolactin levels in rats, rhesus monkeys and humans [see (3,23,49,50) for review]. Opioid antagonists usually reduce prolactin levels in male rhesus monkeys (14,16,24), but both increases and decreases in prolactin levels have been reported in female rhesus monkeys (24,39). Under the conditions of the present study, nalmefene (0.01 and 0.10 mg/kg) did not significantly increase or decrease prolactin in comparison to baseline levels in male rhesus monkeys. In contrast, nalmefene (0.5 or 5.0 mg/kg, IV) significantly increased prolactin levels in normal rhesus females but naloxone had no effect (39). Naloxone (0.05 to 2.0 mg/kg) significantly reduced prolactin levels in male rhesus monkeys (14,16), whereas naltrexone (0.25, 0.5, 1.00 mg/kg, IV) significantly decreased prolactin levels in both male and midluteal female rhesus monkey at some doses, but these effects were not naltrexone dose dependent (24). Interestingly, neither nalmefene nor naloxone had any effect on prolactin in ovariectomized monkeys (39). Thus, no consistent pattern of opioid antagonist effects on prolactin in rhesus monkeys has been reported.

Nalmefene and prolactin pulsatile release. The present study differs from previous preclinical reports of the effects of opioid antagonists on prolactin in that pulsatile release patterns of prolactin were quantified by Cluster analysis techniques (41). However, examination of the peak dimensions (interpeak interval, peak width, peak height) and valley dimensions (valley width, level, and nadir) showed no significant differences from the placebo condition. Moreover, there were no consistent or statistically significant nalmefene dose-dependent changes in prolactin peak or valley characteristics. These findings agree with a previous clinical study in which nalmefene (10 mg., PO) did not change prolactin pulse frequency or amplitude in normal men (17). These data converge to suggest that intravenous nalmefene, over the dose range studied, does not modulate prolactin release patterns in male rhesus monkeys or in human males.

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