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BRIEF COMMUNICATION

Use of Naltrexone as a Provocative Test for Hypothalamic-Pituitary Hormone Function

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MENDELSON, J H , N K MELLO, P CRISTOFARO A SKUPNY AND J ELLINGBOE *Use of naltrexone as a provocative test for hypothalamic-pituitary hormone function* PHARMACOL BIOCHEM BEHAV **24**(2) 309–313, 1986 —Naltrexone (50 mg) administration to normal adult women during the early follicular phase of the menstrual cycle (day 1 to day 4 following onset of menstruation) induced a significant elevation in plasma LH, prolactin ACTH and cortisol levels Orally administered naltrexone appears to be a safe and effective compound for assessing function of the hypothalamic–anterior pituitary axis in women

Naltrexone Hypothalamic-anterior-pituitary hormone function Menstruation

NALTREXONE is currently utilized in conventional medical practice as a pharmacologic adjunct for the treatment of opioid dependence However, we have observed that naltrexone also increases plasma LH levels in normal drug-free adult men [12,14] Subsequent studies have shown that the opioid antagonist, naloxone, stimulates release of pituitary gonadotropins in normal men and women [15] Naltrexone has greater potency and a longer duration of action than naloxone Naltrexone has no opiate agonist properties, induces no significant adverse side effects and can be safely administered orally to men and women

Assessment of anterior pituitary hormone function has been greatly facilitated by the availability of synthetic analogues of hypothalamic releasing hormones TRH and GnRH are routinely employed as specific provocative tests for assessment of pituitary hormonal function [19] Recently synthetic ovine CRH has been employed for assessing ACTH release from the pituitary [4, 5, 18] Simultaneous or sequential administration of hypothalamic releasing hormones has also been employed for determination of pituitary hormone function [3, 7, 11, 16] Very recently, Sheldon and his associates described a rapid sequential intravenous administration procedure utilizing four hypothalamic releasing hormones Sheldon *et al* [20] believe that their combined anterior pituitary function test safely and effectively measures pituitary ACTH, FSH, LH, TSH and PRL secretory function

To date no provocative tests for assessing anterior pituitary hormone function have been possible without intramuscular and/or intravenous hypothalamic releasing hormone administration This report describes the use of orally administered naltrexone as a provocative stimulus for assessing multiple anterior hormone function

METHOD

Four adult women between the ages of 22 and 28 provided informed consent for participation in these studies All women had a past history of normal menstrual cycle function None had been pregnant and none used contraceptive medication or intrauterine devices All had normal physical, mental status, blood chemistry, urinanalysis and blood hemogram studies No subject had any past history of alcohol or drug abuse and none were using any medications at the time of the study Urine screens for analgesic, stimulant, depressant and other psychoactive drugs were negative

The study was carried out during the follicular phase of the menstrual cycle Subjects reported to the laboratory at 9 a m following a twelve hour fast An indwelling catheter was placed in the antecubital vein and connected to a slow intravenous infusion of 5% dextrose and saline Subjects were recumbent throughout the study, and were not permitted to eat solid foods, smoke or drink beverages containing caffeine However, they could drink noncarbonated fruit juice and they were also able to read or watch television

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Following collection of four consecutive blood samples at thirty minute intervals, subjects ingested one 50 mg tablet of naltrexone hydrochloride Blood samples were collected at consecutive thirty-minute intervals for 240 minutes following naltrexone administration

Plasma samples were aliquoted and frozen (−70°C) for subsequent analysis of LH, prolactin and cortisol

Plasma LH and prolactin levels were measured in duplicate by a double-antibody radioimmunoassay (RIA) procedure similar to that described by Midgley [13] Antisera and reference preparations (LER-907 for LH, hPRL-RP-1 for prolactin) were provided by the National Hormone and Pituitary Program (University of Maryland School of Medicine), supported by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases Iodine-125-labeled human LH and prolactin were purchased from Cambridge Medical Diagnostics (Billerica, MA) Results are expressed in terms of the LH and prolactin reference preparations Assay sensitivities were 3 4 and 3 1 ng/ml for the LH and prolactin assays, respectively Intra- and interassay coefficients of variation (CVs) were 5 8 and 5 4 percent for the LH assay and 6 8 and 14 percent for the prolactin assay

Plasma ACTH concentrations were measured in duplicate using a direct, double antibody radioimmunoassay kit purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA) Assay sensitivity was 10 pg/ml Intra- and interassay coefficients of variation were 8 1 and 22 4 percent, respectively

Cortisol was determined in duplicate plasma samples by a direct double-antibody RIA method that did not require solvent extraction, using a kit purchased from Clinical Assays (Cambridge, MA) The assay sensitivity was less than 2 ng/dl Intra- and interassay CVs were 3 4 and 9 4 percent

RESULTS

Figure 1 shows LH values for two subjects (1 and 2) studied during the early follicular phase of the menstrual cycle (top panel) and two subjects (3 and 4) studied during the mid and late follicular phase (bottom panel) Baseline LH levels prior to naltrexone administration for subjects (1 and 2) studied during the early follicular phase ranged between 8 to 18 ng/ml Peak LH values were detected 210 minutes following naltrexone intake Peak LH increments above mean baseline levels were 63 ng/ml for subject 1 and 72 ng/ml for subject 2 (500% and 1300% increments, respectively)

Subject 3 was studied during the mid follicular phase and had baseline LH values averaging 30 ng/ml Peak LH values were detected 180 minutes following naltrexone administration The peak increment in LH values for this subject was 33 ng/ml (95% increase above baseline values)

Subject 4 studied during the late follicular phase of the menstrual cycle had baseline LH levels averaging 90 ng/ml Peak LH levels following naltrexone administration were detected at 210 minutes LH levels increased by 55 ng/ml (50% increase above baseline values)

Prolactin levels prior to and following naltrexone administration for subjects studied during the early, mid and late follicular phase of the menstrual cycle are shown in Fig 2 Baseline prolactin levels for the subjects studied during the early follicular phase (top panel Fig 2) ranged between 3 and 17 ng/ml Following naltrexone administration, peak prolactin levels were detected at 120 to 210 minutes The peak increment in prolactin levels was 18 to 20 ng/ml or 250 to 600% above baseline values

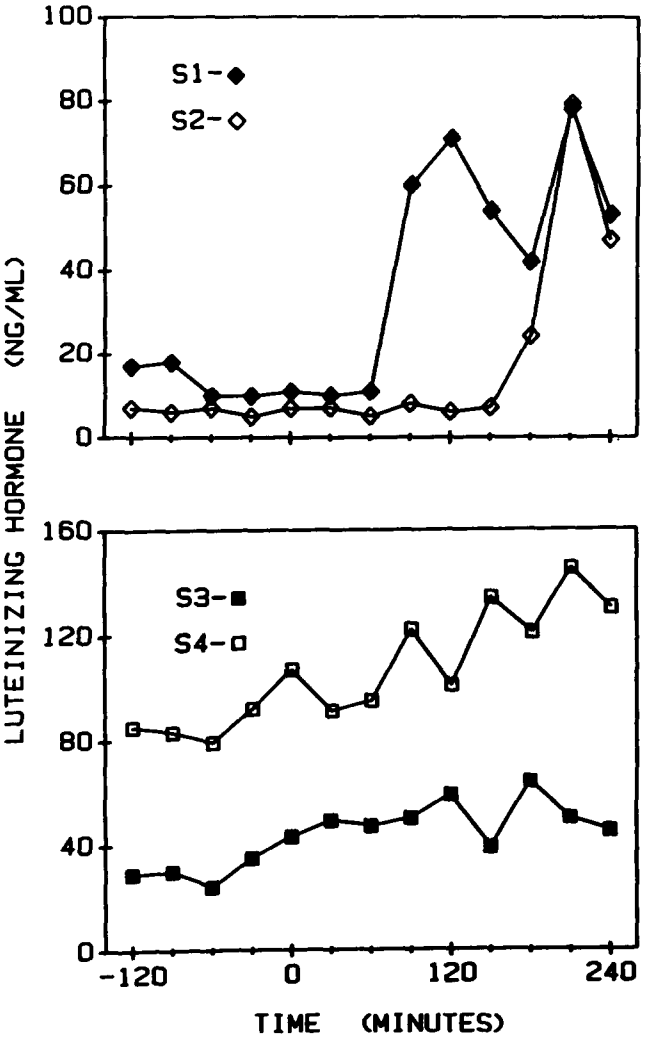

FIG 1 Luteinizing hormone levels prior to and following naltrexone administration at 0 time Top panel shows LH values for two subjects (S-1, S-2) studied during the early follicular phase of the menstrual cycle Bottom panel shows LH values for two subjects (S-3, S-4) studied during the mid and late follicular phase of the menstrual cycle

Subjects studied during the mid and late follicular phase of the menstrual cycle (bottom panel Fig 2) had higher baseline prolactin levels than those women studied during the early follicular phase Naltrexone administration induced an increase in prolactin levels 180 to 210 minutes following drug intake Prolactin levels increased 16–18 ng/ml or approximately 100% above baseline values

Figure 3 shows plasma ACTH levels for subjects studied during the early, mid and late follicular phases of the menstrual cycle During the early follicular phase (top panel Fig 3), ACTH values ranged between 5 and 22 pg/ml Peak ACTH levels following naltrexone administration were detected 120 to 140 minutes following drug intake Plasma ACTH levels increased by an average of 15 pg/ml or 100 to 200% over baseline values During the mid and late follicular phase of the menstrual cycle (bottom panel Fig 3), baseline ACTH levels ranged between 10–48 pg/ml Subject 3 had an

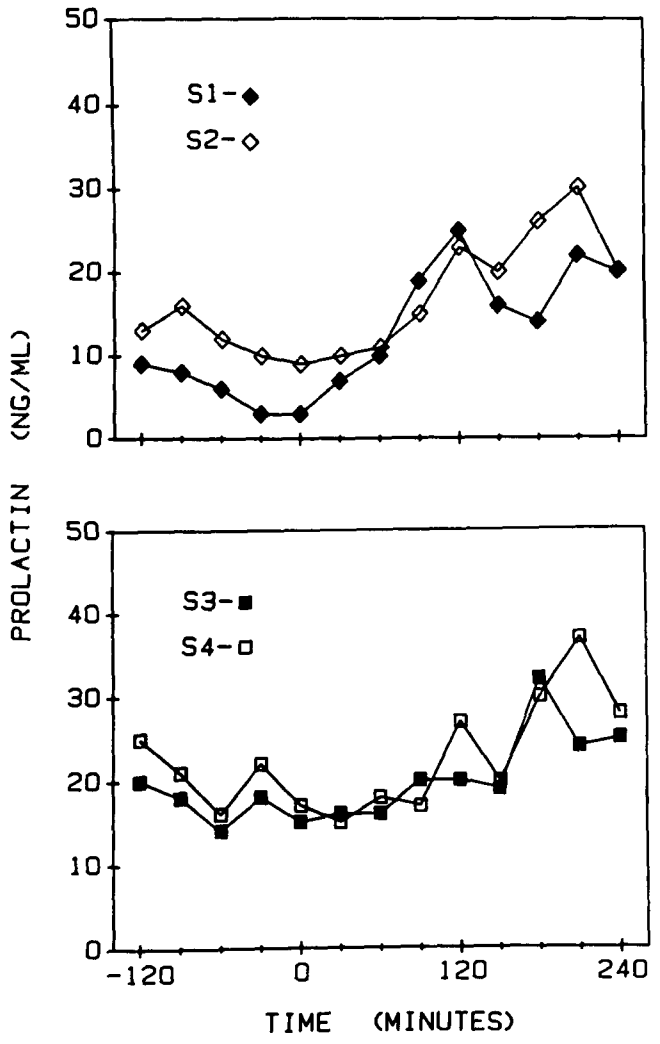

FIG 2 Prolactin levels prior to and following naltrexone administration at 0 time Top panel shows prolactin values for two subjects (S-1, S-2) studied during the early follicular phase of the menstrual cycle Bottom panel shows prolactin values for two subjects (S-3, S-4) studied during the mid and late follicular phase of the menstrual cycle

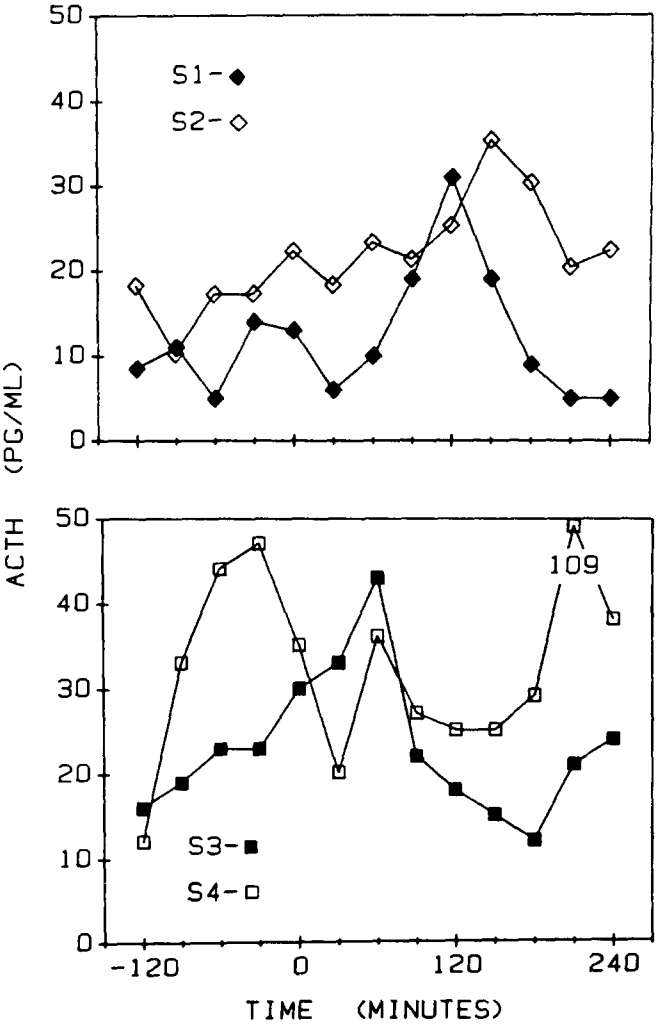

FIG 3 ACTH levels prior to and following naltrexone administration at 0 time Top panel shows ACTH values for two subjects (S-1, S-2) studied during the early follicular phase of the menstrual cycle Bottom panel shows ACTH values for two subjects (S-3, S-4) studied during the mid and late follicular phase of the menstrual cycle

increase in ACTH levels which peaked at +60 minutes Subject 4 had an ACTH increment at +210 minutes There was considerable variation in ACTH levels for both subjects studied during the mid and late follicular phase of the menstrual cycle

Figure 4 shows plasma cortisol levels for subjects studied during the early, mid and late follicular phase of the menstrual cycle During the early follicular phase (top panel Fig 4), cortisol values ranged between 6 and 13 ng/dl. Peak cortisol levels following naltrexone administration were detected 120 to 180 minutes following drug intake Plasma cortisol levels increased by an average of 8 ng/dl or 100% over baseline values During the mid and late follicular phase of the menstrual cycle (bottom panel Fig 4), baseline cortisol levels ranged between 9 and 13 ng/dl Peak cortisol levels following naltrexone administration were detected 60 to 180 minutes following drug intake Increments in plasma cortisol levels ranged between 4 to 5 ng/dl over baseline values (45% increment)

DISCUSSION

Naltrexone-induced LH stimulation observed in women studied during the early follicular phase of the menstrual cycle was of greater magnitude than LH increments reported following administration of the standard dose of synthetic luteinizing hormone releasing hormone (Factrel 100 m IM) [2] Administration of synthetic luteinizing hormone releasing hormone (Factrel 100 m IM) induces a peak increment in LH levels in women ranging between 100 and 500% above baseline values [2] The magnitude of the LH and prolactin response for women studied during the early follicular phase of the menstrual cycle was greater than the magnitude of LH and prolactin responses induced by the combined anterior

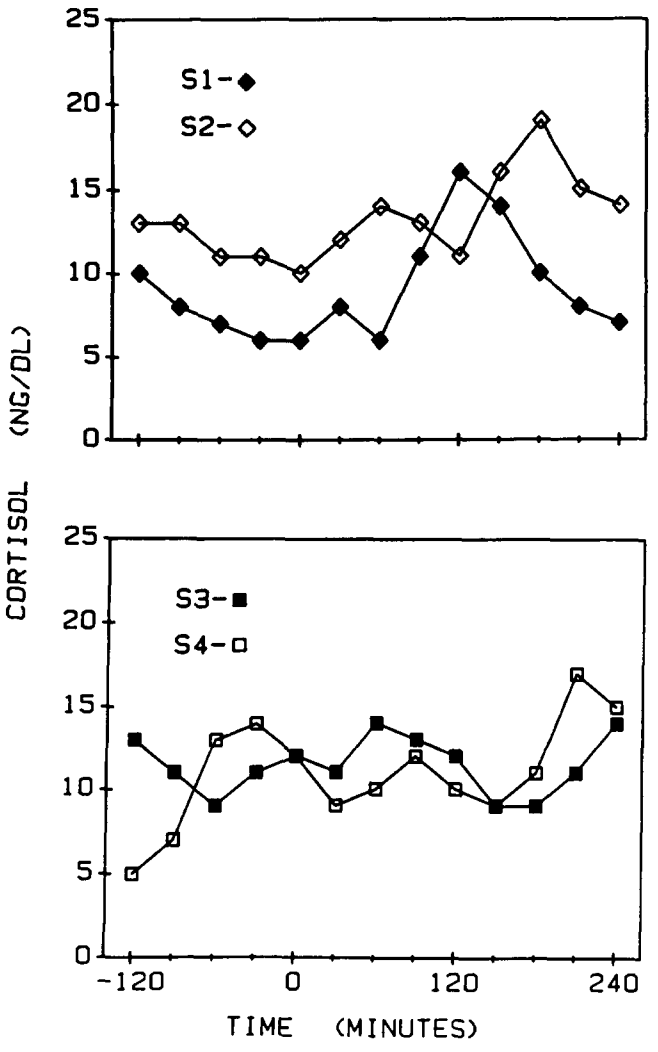

FIG 4 Cortisol levels prior to and following naltrexone administration at 0 time Top panel shows cortisol values for two subjects (S-1, S-2) studied during the early follicular phase of the menstrual cycle Bottom panel shows cortisol values for two subjects (S-3, S-4) studied during the mid and late follicular phase of the menstrual cycle

pituitary function test described by Sheldon and his colleagues [20]

The increase in ACTH levels following naltrexone administration to subjects studied during the early follicular phase of the menstrual cycle was concordant with the increase in plasma cortisol levels Wide variations in baseline plasma cortisol ACTH levels were found in subjects studied during the mid and late follicular phases of the menstrual cycle

Data obtained in this study differ from the observations of Snowden *et al* [21] that opioid antagonists have maximal stimulatory effects on gonadotropin and prolactin secretion during the *luteal* phase of the menstrual cycle However, Snowden *et al* [21] used naloxone instead of naltrexone and they also did not study women during the very early follicular phase of the menstrual cycle

There has been continuing controversy about variations in sensitivity to LHRH at different phases of the menstrual cycle LHRH is usually administered during the early follicular phase for clinical diagnostic evaluations But it has been reported that sensitivity to LHRH increases during the mid to late follicular phase and is maximal during the mid-cycle surge [9] Fern and co-workers [6] reported a significantly enhanced LH response to LHRH (0 2–100 mg) at mid-cycle in rhesus females in comparison to the early, middle or late follicular phase LHRH (20–100 mg) also stimulated higher LH responses during the luteal phase than during the follicular phase [6] Relative insensitivity to LHRH (10–80 mg) during the follicular phase was also observed in rhesus females by Hobson and Fuller [8] However, other investigators have reported no difference in response to LHRH stimulation during the follicular and luteal phase in women [17] and rhesus females [1,10] Many procedural differences including the type of LHRH preparation employed, the LHRH dose and mode of administration (bolus vs continuous infusion) as well as frequency of sample collection preclude any simple resolution of these conflicting observations

Our observations that naltrexone administration induces a large increment in plasma LH, prolaction, ACTH and cortisol levels during the early follicular phase of the menstrual cycle suggests that naltrexone may be employed as a provocative test for assessing hypothalamic-pituitary function Currently available combined pituitary tests require parenteral administration of a combination of synthetic hypothalamic releasing hormones These tests permit determination of pituitary response to hypothalamic releasing hormones but do not directly evaluate hypothalamic releasing hormone function *plus* pituitary response Naltrexone administration on the other hand, stimulates pituitary hormone secretion as a consequence of the drug's effect on neuronal modulation of endogenous hypothalamic releasing hormones Since maximal hormonal responses were observed during the early follicular phase (days 1 to 3 of menstruation) accurate scheduling of naltrexone provocative tests would be possible for optimal clinical feasibility Thus orally administered naltrexone may be a safe and effective drug for assessing function of the hypothalamic-anterior-pituitary axis in women

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