

Sex Differences in Salivary Cortisol Levels Following Naltrexone Administration¹

LAURA COUSINO KLEIN²

*Department of Biobehavioral Health
The Pennsylvania State University*

LARRY D. JAMNER, JANEL ALBERTS, MATTHEW D. ORENSTEIN,

AND LINDA LEVINE

University of California, Irvine

HOYLE LEIGH

University of California, San Francisco

and

Fresno Veterans Affairs Medical Center

Effects of endogenous opioid peptide blockade by naltrexone on salivary cortisol levels were examined in healthy men ($n = 8$) and women ($n = 6$). Participants received naltrexone (100 mg) during one laboratory session and a placebo pill during another session. Drug order was counterbalanced across participants. Saliva samples were collected 24 hr after each pill was administered. Among women, salivary cortisol levels significantly increased following naltrexone administration compared with a placebo pill. Naltrexone administration did not alter salivary cortisol levels in men. Results suggest sex differences in neuroendocrine sensitivity to opioid blockade, a finding that may hold significance with regard to the treatment of alcohol addiction with naltrexone.

The endogenous opioid peptide system has several physiological roles including pain inhibition, appetite regulation, and modulation of the stress response

¹This work was conducted at the University of California, Irvine, and was supported partially by a University of California, Irvine, career development award (447614-09503-8) granted to the first author. This study was supported partially by a Merit Review grant awarded to H. Leigh by the Department of Veterans Affairs. Additional support for biochemical assays was provided by NIMH grant 5T32MH19958-03. The procedures followed in this research were reviewed and approved by University of California, Irvine, human-subjects committees and were performed in accordance with human-use ethical standards. These findings were presented at the 38th meeting of the Society for Psychophysiological Research. We thank Larry Cahill, Elissa Epel, and Douglas Granger for their assistance, as well as William Lovallo and Regan A. R. Gurung for their comments.

²Correspondence concerning this article should be addressed to Laura Cousino Klein, Department of Biobehavioral Health, Penn State University, 315 East Health and Human Development, University Park, PA 16802-6509. e-mail: LXX18@psu.edu

through the hypothalamic-pituitary-adrenomedullary (HPA) axis. Endogenous opioid peptides (EOPs) are derived from proopiomelanocortin (POMC), which also is the precursor for adrenocorticotropin hormone (ACTH). Both ACTH and EOPs are released from the anterior pituitary, and ACTH stimulates release of glucocorticoids (e.g., cortisol) from the adrenal glands. EOP blockade by opioid antagonists (e.g., naloxone and naltrexone) also has important effects on HPA-axis functioning.

Earlier studies report that the administration of naloxone, a short-acting opioid antagonist used in the treatment of opioid overdose, increases plasma cortisol levels in men (Grevert, Albert, Inturrisi, & Goldstein, 1983; Grossman, Gaillard, McCartney, Rees, & Besser, 1982; Grossman, Moulton, Cunnah, & Besser, 1986). It is believed that naloxone increases cortisol release by suppressing the ACTH inhibitory effects of EOPs in humans (Grossman et al., 1982; Morley, Baranetsky, Wingert, Carlson, & Hershman, 1980; Volvavka, Cho, Mallya, & Bauman, 1979). Our review of the literature on humans suggests that the reported effects of opioid antagonism on HPA-axis activity are based primarily on the administration of naloxone, which produces short-term opioid receptor blockade (i.e., 90 min). It is possible that the long-term blockade of endogenous opioid receptors has different physiological consequences than does the acute, short-term blockade by naloxone.

Naltrexone is a long-acting opioid antagonist that works primarily on μ -opioid receptors to block the binding of EOPs (e.g., β -endorphin) and exogenous opiates (e.g., morphine) to these receptors (Jaffe & Martin, 1990). Naltrexone has a plasma half-life of about 10 hr (depending on dose administration), and it is extensively metabolized into the primary active metabolite, 6- β -naltrexol, by the liver. Because of the pharmacokinetic properties of naltrexone and its metabolites, a single 100-mg oral dose of naltrexone will block μ -opioid receptors for at least 48 hr (Way, Fields, & Way, 1998). One study indicated that μ -opioid receptors in the brain remained blocked up to 100 hr after the administration of a dose as low as 50 mg/day (Lee et al., 1988). This point is noteworthy given that naltrexone recently received Food and Drug Administration (FDA) approval as a pharmacologic treatment for alcoholism. In these clinical cases, men and women are dosed daily with 50 to 100 mg of naltrexone, over a course of several months, to curb alcohol cravings and decrease alcohol consumption (for review, see Weinrieb & O'Brien, 1997). Although this antagonist is used as a long-term treatment for alcoholism (Litten & Allen, 1998, 1999), little is known about the long-term effects of endogenous opioid blockade on HPA-axis activity in healthy volunteers. Empirical studies on the HPA-axis effects of naltrexone examine neuroendocrine changes for up to 12 hr after drug administration; clinically, however, naltrexone is administered in doses designed to block μ -opioid receptors for up to 48 to 72 hr (depending on dose) to treat alcoholism. Our review of the literature found only one published report of the effects of naltrexone on

Table 1

Demographic Characteristics of Sample (Means \pm Standard Error of Mean)

	Women ($n = 8$)	Men ($n = 8$)
Age (years)	33.38 \pm 4.25	29.75 \pm 2.98
Education (years)	14.25 \pm 1.98	15.50 \pm 1.77
Weight (pounds)	137.50 \pm 3.71	174.25 \pm 9.70
Height (inches)	65.00 \pm 0.50	70.38 \pm 0.76
Body Mass Index	22.89 \pm 0.63	24.70 \pm 1.22
Ethnicity		
Caucasian	75.0% ($n = 6$)	87.5% ($n = 7$)
Asian American	12.5% ($n = 1$)	12.5% ($n = 1$)
African American	12.5% ($n = 1$)	0%
Marital status		
Single (never married)	37.5% ($n = 3$)	37.5% ($n = 3$)
Married	50.0% ($n = 4$)	50.0% ($n = 4$)
Divorced	12.5% ($n = 1$)	12.5% ($n = 1$)

neuroendocrine function in humans (Mendelson, Ellingboe, Keuhnle, & Mello, 1979). However, naltrexone's effect on cortisol levels was not examined, and women were not included in the study. Therefore, the present study examined the effects of naltrexone administration on salivary cortisol (i.e., free cortisol) responses 24 hr later in men and women as a first step to understanding the longer time course of the HPA-axis effects of naltrexone administration.

Method

Participants

Participants included 16 healthy nonsmokers (8 women and 8 men) between the ages of 18 and 45 (mean = 31.56 years) who were recruited from the University of California, Irvine, campus and local community through e-mail and flyers as part of a larger ongoing project investigating the effects of naltrexone on ambulatory physiological functioning. The men and women were similar in age, years of education, body mass index, ethnicity, and marital status (Table 1). Upon screening, individuals who reported health problems, histories of alcohol or drug abuse, or current medication use were excluded. Volunteers were paid for their participation in the larger project, which lasted for 3 days.

Menstrual cycle phase was not controlled for in the present experiment, and women on oral contraceptives (OC) also were included in the study. Although

menstrual cycle and OC use influence salivary free cortisol responses to stress in women, basal salivary free cortisol levels (i.e., free cortisol levels upon awakening in the morning) do not differ among women in the luteal or follicular phases of the menstrual cycle or among those using oral contraceptives (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

Study Design

The study incorporated a within-subject double-blind randomized design. In one session, participants ingested an oral dose (100 mg) of naltrexone hydrochloride (Trexan[®]; DuPont Pharma, Delaware). In the other session, they ingested an inert placebo pill (DuPont Pharma, Delaware). The naltrexone dose was selected on the basis of current research that indicates effective opioid blockade in male and female alcohol-dependent patients at doses ranging from 20 mg/day to 100 mg/day (Litten & Allen, 1998; Way et al., 1998). When naltrexone doses were adjusted for body weight (kg), women received 1.61 ± 0.04 mg naltrexone/kg and men received 1.29 ± 0.07 mg naltrexone/kg. Drug conditions were counterbalanced across participants.

Procedure

The participants completed an orientation session that was followed by two drug sessions, which were 1 week apart. During the orientation and after informed consent was received, the participants were administered a health-history interview to ensure their eligibility for the study, which included the stipulation that they have no history of alcohol or substance abuse. In addition, a blood sample was drawn and assayed for healthy liver enzyme activity. Women were given pregnancy tests at the beginning of each experimental session to ensure that they were not pregnant before receiving the medication.

The participants arrived at the lab where they were given a pill to swallow with a glass of water. They were observed in the lab for 90 min, and then they were asked to come back the following morning to complete a variety of tasks as part of a larger study. The participants were asked to abstain from prescription or over-the-counter medications, alcohol, and nicotine products 24 hr before arriving at the lab and for 3 days after consuming the pill. The participants also were asked to limit their caffeine intake to two servings of caffeine per day (i.e., two 8 oz. cups of coffee or caffeinated sodas).

Saliva Collection

Saliva samples were collected in the laboratory the next morning when participants arrived for the laboratory session. Samples were collected 24 hr

after each pill was administered (i.e., naltrexone and placebo), between 8:00 a.m. and 10:30 a.m. For each participant, collection times were similar across sessions to control for potential circadian changes in cortisol levels (Grossman et al., 1982; Ockenfels et al., 1995). Salivary cortisol levels were assessed 24 hr after the pills were administered to examine the long-term effects of endogenous opioid blockade. Previous studies indicate that a 100-mg dose of naltrexone results in μ -opioid receptor blockade for up to 100 hr after administration (Lee et al., 1988).

After relaxing and completing questionnaires about the previous 24-hr period, the participants were asked to produce a saliva sample by chewing a piece of gum (Trident™ sugarless spearmint gum) for 2 min (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). Next, they were asked to roll a cotton swab in their mouths for an additional 2 min before being instructed to place the swab into a Salivette® saliva collection tube (Sarstedt, Inc., North Carolina). Samples immediately were placed on ice and then frozen (-70 degrees Celsius) for later assessment of cortisol by radioimmunoassay (Coat-a-Count® assay; Diagnostic Products Corporation, Los Angeles, California). Samples were shipped on dry ice by overnight delivery to the Behavioral Endocrinology Laboratory at The Pennsylvania State University, University Park campus, where the cortisol assays were conducted. All samples were tested in duplicate in a minimum number of assay batches. Values used in data analyses were the averages of the duplicate tests. The assay had a lower limit of sensitivity of 0.03 μ g/dl, with an average inter- and intra-assay covariance of less than 10% and 5%, respectively. Individual samples with duplicate tests that varied by more than 5% were repeated in subsequent runs.

Treatment of Data and Statistical Analyses

Two samples (1 placebo day and 1 naltrexone day) from two different female participants were lost during sample processing. Therefore, these women are not included in the analyses. Natural logarithmic transformations were applied to the raw data because the raw cortisol values were not normally distributed. Because of the variability in cortisol responses, previous investigations of salivary cortisol have applied logarithmic transformations to salivary cortisol data (e.g., Ockenfels et al., 1995). In the present study, this transformation resulted in a normal distribution of the data; therefore, all reported analyses are based on these logarithmic-transformed values. However, raw cortisol values (\pm standard error of the mean) were used to graph the data for clarity.

Because women received a greater naltrexone dosage than did men, $F(1, 13) = 11.87, p < .05$, the following analyses were conducted using dosage as a covariate. However, naltrexone dosage did not emerge as a significant covariate in any of the analyses; therefore, analyses without the covariate are reported.

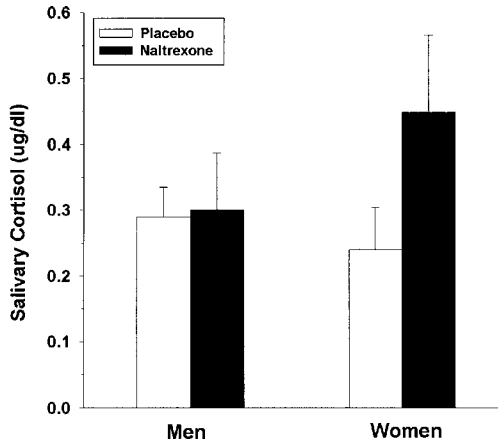


Figure 1. Effects of naltrexone on salivary cortisol levels ($\mu\text{g/dl}$) in men and women. Error bars are equal to the standard error of the mean.

Results

Figure 1 presents mean salivary cortisol levels following naltrexone and placebo administration in men and women. A repeated-measures analysis of variance (ANOVA) was conducted to evaluate salivary cortisol responses to naltrexone compared with placebo cortisol levels. The results revealed a significant drug by sex interaction, $F(1, 12) = 4.71, p = .05$. Specifically, among women, salivary cortisol levels were significantly higher in response to naltrexone compared with the placebo condition, $F(1, 5) = 7.04, p < .05$. In contrast, naltrexone administration did not alter salivary cortisol levels in men compared with the placebo condition. Separate ANOVAs indicated that there were no sex differences in cortisol levels under naltrexone or placebo conditions.

Discussion

The present study examined the effects of endogenous opioid blockade by naltrexone on salivary cortisol responses in men and women. The results suggest that naltrexone administration significantly increases salivary cortisol 24 hr after administration in women but not in men. This finding for men is surprising in light of previous reports of increases in plasma cortisol responses among men shortly following administration of naloxone and naltrexone (Grevert et al., 1983; Grossman et al., 1982, 1986).

There are several pharmacokinetic and pharmacodynamic explanations for why the present results may differ from the current literature. Although pharmacologically similar to naloxone, naltrexone is two to eight times more

potent than naloxone (Blumberg & Dayton, 1973; Verebely & Mule, 1975), and naltrexone's actions last hours longer than do naloxone's actions (Martin, Jasin-ski, & Mansky, 1973). It is possible that long-term blockade of endogenous opioid receptors resulted in an attenuation or habituation of the cortisol response among men but not among women, suggesting a different dose-response curve for men and women. A recent study observed higher μ -opioid receptor binding among women (Zubieta, Dannals, & Frost, 1999), suggesting that the observed increase in cortisol in the present study was a result of greater antagonism of the central opioid system stemming from increased naltrexone binding in women. These results add to an increasing body of literature on sex differences in opioid sensitivity and opioid-hormone interactions in humans and animals (e.g., Barbarino et al., 1987; Crowley, 1988; Fernandez et al., 1999; Klein, Popke, & Grunberg, 1998; Kreek, Schluger, Borg, Gunduz, & Ho, 1999; Schlenker, Martin, Lin, & Eglund, 1997; Zubieta et al., 1999). Interestingly, there are no published reports on sex differences in the pharmacokinetics of naltrexone per se, and the naltrexone manufacturer, DuPont Pharma (Wilmington, DE), reports no indication of gender differences in the pharmacokinetics of naltrexone in company records (L. Lupo, personal communication, November 14, 2000). Thus, the issue surrounding sex differences in naltrexone metabolism, distribution, absorption, and excretion, as well as the subsequent impact on neuroendocrine functioning, remains unclear. The present results suggest that future studies need to directly address this question.

Another point directly related to understanding the observed sex difference in cortisol responses to naltrexone is that perhaps higher naltrexone dosages among women contributed to the observed increase in cortisol levels. We addressed this question by conducting a separate analysis of covariance (ANCOVA) on cortisol levels with mean naltrexone dosage (i.e., mg naltrexone/kg body weight) as the covariate, and we found that the main effect for naltrexone on cortisol levels remained. Although naltrexone is administered clinically in 50- or 100-mg doses to patients without adjusting for body weight, a direct comparison of cortisol responses to naltrexone in men and women needs to be made with naltrexone amounts adjusted for body weight.

A final explanation for the differences between previously reported results and the present findings is that cortisol levels were assessed 24 hr after the administration of naltrexone. No other studies have evaluated these long-term effects of opioid blockade on HPA-axis activity. These findings indicate that, at the dose administered, women demonstrate greater HPA-axis sensitivity to endogenous opioid blockade than do men. Given that this dose (100 mg) is similar to that used in alcohol addiction treatment, this finding may have important implications for chronic alcohol treatment with naltrexone.

The present results should be approached with caution, however, because of the small sample size and single saliva sample collection procedures. The use of

a single saliva sample method prevents an assessment of time effects on cortisol responses beyond the influence of naltrexone. Furthermore, situational factors could account for cortisol differences between men and women, but they were not captured because of the small window of cortisol assessment. These limitations are important in light of the relevance of the present findings to understanding interactions between the HPA-axis and EOP systems. The present experiment is a first step in developing an understanding of the time-course neuroendocrine effects of naltrexone administration. The results suggest that sex differences may contribute to naltrexone's efficacy in the treatment of alcoholism. They also indicate that additional investigations on sex differences in naltrexone's neuroendocrine effects are necessary.

References

- Barbarino, A., De Marinis, L., Mancini, A., D'Amico, C., Passeri, M., Zuppi, P., Sambo, P., & Tofani, A. (1987). Sex-related naloxone influence on growth hormone-releasing hormone-induced growth hormone secretion in normal subjects. *Metabolism*, **36**, 105-109.
- Blumberg, H., & Dayton, H. B. (1973). Naloxone, naltrexone, and related noroxymorphones. *Advances in Biochemistry and Psychopharmacology*, **8**, 33-43.
- Crowley, W. R. (1988). Sex differences in the responses of hypothalamic luteinizing hormone-releasing hormone and catecholamines systems to ovarian hormones and naloxone: Implications for sexual differentiation of luteinizing hormone secretion in rats. *Brain Research*, **461**, 314-321.
- Fernández, B., Antelo, M. T., Guaza, C., Alberti, I., Pinillos, M. L., & Viveros, M. P. (1999). Naltrindole administration during the preweaning period and manipulation affect adrenocortical reactivity in young rats. *Developmental Brain Research*, **112**, 135-137.
- Grevert, P., Albert, L. H., Inturrisi, C. E., & Goldstein, A. (1983). Effects of eight-hour naloxone infusions on human subjects. *Biological Psychiatry*, **18**, 1375-1392.
- Grossman, A., Gaillard, R. C., McCartney, P., Rees, L. H., & Besser, G. M. (1982). Opiate modulation of the pituitary-adrenal axis: Effects of stress and circadian rhythm. *Clinical Endocrinology*, **17**, 279-286.
- Grossman, A., Moul, P. J. A., Cunnah, D., & Besser, G. M. (1986). Different opioid mechanisms are involved in the modulation of ACTH and gonadotrophin release in man. *Neuroendocrinology*, **42**, 357-360.
- Jaffe, J. H., & Martin, W. R. (1990). Opioid analgesics and antagonists. In A. G. Gilman, T. W. Rall, A. S. Nies, P. Taylor (Eds.), *Goodman and Gilman's: The pharmacological basis of therapeutics* (8th ed., pp. 485-521). New York, NY: McGraw Hill.

- Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine*, **61**, 154-162.
- Klein, L. C., Popke, E. J., & Grunberg, N. E. (1998). Sex differences in effects of opioid blockade on stress-induced freezing behavior. *Pharmacology, Biochemistry, & Behavior*, **61**, 413-417.
- Kreek, M. J., Schluger, J., Borg, L., Gunduz, M., & Ho, A. (1999). Dynorphin A₁₋₁₃ causes elevation of serum levels of prolactin through an opioid receptor mechanism in humans: Gender differences and implications for modulation of dopaminergic tone in the treatment of addictions. *The Journal of Pharmacology and Experimental Therapeutics*, **288**, 260-269.
- Lee, M. C., Wagner, H. N., Tanada, S., Frost, J. J., Bice, A. N., & Dannals, R. F. (1988). Duration of occupancy of opiate receptors by naltrexone. *Journal of Nuclear Medicine*, **29**, 1207-1211.
- Litten, R. Z., & Allen, J. P. (1999). Medications for alcohol, illicit drug, and tobacco dependence. An update of research findings. *Journal of Substance Abuse Treatment*, **16**, 105-112.
- Litten, R. Z., & Allen, J. P. (1998). Advances in development of medications for alcoholism treatment. *Psychopharmacology*, **139**, 20-33.
- Martin, W. R., Jasinski, D. R., & Mansky, P. A. (1973). Naltrexone: An antagonist for the treatment of heroin dependence. *Archives of General Psychiatry*, **28**, 784-791.
- Mendelson, J. H., Ellingboe, J., Keuhle, J. C., & Mello, N. K. (1979). Effects of naltrexone on mood and neuroendocrine function in normal adult males. *Psychoneuroendocrinology*, **3**, 231-236.
- Morley, J. E., Baranetsky, N. G., Wingert, T. D., Carlson, H. E., & Hershman, J. M. (1980). Endocrine effects of naloxone-induced opiate receptor blockade. *Journal of Clinical Endocrinology and Metabolism*, **50**, 251-257.
- Ockenfels, M. C., Porter, L., Smyth, J., Kirschbaum, C., Hellhammer, D. H., & Stone, A. A. (1995). Effect of chronic stress associated with unemployment in salivary cortisol: Overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosomatic Medicine*, **57**, 460-467.
- Schlenker, E. H., Martin, D. S., Lin, X. M., & Eglund, M. C. (1997). Naloxone microinjected into the arcuate nucleus has differential effects on ventilation in male and female rats. *Physiology & Behavior*, **62**, 531-536.
- Schwartz, E. B., Granger, D. A., Susman, E. J., Gunnar, M. R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development*, **69**, 1503-1513.
- Verebely, K., & Mule, S. J. (1975). Naltrexone pharmacology, pharmacokinetics, and metabolism: Current status. *American Journal of Drug and Alcohol Abuse*, **2**, 357-363.

- Volvavka, J., Cho, D., Mallya, A., & Bauman, L. (1979). Naloxone increases ACTH and cortisol levels in man. *New England Journal of Medicine*, **300**, 1056-1057.
- Way, W. L., Fields, H. L., & Way, E. L. (1998). Opioid analgesics and antagonists. In B. G. Katzung (Ed.), *Basic and clinical pharmacology* (7th ed., pp. 496-515). New York, NY: Appleton & Lange.
- Weinrieb, R. M., & O'Brien, C. P. (1997). Naltrexone in the treatment of alcoholism. *Annual Review of Medicine*, **48**, 477-487.
- Zubieta, J., Dannals, R. F., & Frost, J. J. (1999). Gender and age influences on human brain mu-opioid receptor binding measured by PET. *American Journal of Psychiatry*, **156**, 842-848.