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Sex and opioid maintenance dose influence response to naloxone in opioid-dependent humans: A retrospective analysis

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ABSTRACT

Pooled self-report and physiological data from 32 male and 15 female methadone or *levo*-α-acetyl methadol (LAAM) maintained volunteers were retrospectively analyzed for individual differences in response to naloxone (0.15 mg/70 kg, IM) and placebo at 20 and 40 min post-injection. Males and females were each divided by the median split methadone maintenance dose (MMD, in mg/kg body weight) into high and low MMD groups and MMD was used as a factor in the analyses, along with sex, drug, and time post-drug. Females in the low, but not high, MMD group showed naloxone-induced increases in ratings on the Antagonist and Mixed-Action sub-scales of the Adjective Rating Scale, and the Lysergic acid diethyl amine (LSD) sub-scale of the Addiction Research Center Inventory at 20 min post-injection. Males in the high MMD group showed significant naloxone-induced increases in scores of these measures at both post-injection time-points. In addition, low MMD subjects showed more short-lived naloxone-induced increases on Visual Analogue Scale (VAS) Bad and Any drug effects ratings than high MMD subjects. These results suggest that those on a lower MMD, especially women, experience a more intense, but short-lived, response to naloxone, whereas those on a higher MMD experience a more modest, but longer-lasting effect.

1. Introduction

Even though more than 150,000 persons in the United States are in opiate maintenance treatment, about 40% of whom are female, (Levine et al., 2004; also available at: http://www.oas.samhsa.gov/ADSS/methadone.pdf), few controlled studies have examined how sex or maintenance opiate dose might explain individual differences during opiate withdrawal. Most published studies about opiate maintenance, including a 33-year follow-up of heroin addicts, have examined only males as participants in their cohort (Hser et al., 2001). There is evidence that males and females respond differently to a variety of commonly-used substances including alcohol (Fillmore et al., 1997; Lancaster and Spiegel, 1992), benzodiazepines (Jackson et al., 2005), cocaine (Singha et al., 2000), nicotine (Perkins et al., 2002) and opiates (Zacny, 2001; Zacny and Beckman, 2004). While sex differences in opioid antinociception or opioid-induced analgesia have been studied extensively for a number of different opiates (Barrett, 2006), a paucity of data exists regarding sex-related differences in opiate withdrawal in opioid-dependent humans. Hence, we performed a retrospective analysis of pooled data from five naloxone discrimination studies with the aim of examining potential sex and opiate-dose related differences in response to naloxone in opioid-maintained individuals (Oliveto et al., 1998, 2002, 2003a,b, 2004).

Evidence from animal studies indicates that male and female rodents differ in their response to opiates under several abuse-liability paradigms, including self-administration and other indirect measures of the reinforcing effects of opiates, such as conditioned place preference and locomotor activity (see reviews by Lynch et al., 2002; Carroll et al., 2004). For instance, female rats have been shown to acquire heroin self-administration at a faster rate than male rats (Lynch and Carroll, 1999; Carroll et al., 2002) and female rodents consumed greater amounts of opiates compared to the male rodents (Carroll et al., 2001; Alexander et al., 1978; Hadaway et al., 1979). In addition to consuming greater amounts of fentanyl compared to male rats, female rats were also seen to self-administer greater amounts of fentanyl during experimentally produced periods of chronic stress (Klein et al., 1997). Under a conditioned place preference paradigm, female rats demonstrated a much stronger preference for the place where they were administered morphine than where placebo was administered, compared to male rats (Randall et al., 1998). In addition, female rats were also shown to be less sensitive to the morphine-induced suppression of locomotor activity (Craft et al., 2006).

Similarly, sex-related differences have been demonstrated in the expression of physical dependence and withdrawal in rodents. For instance, naloxone produced greater withdrawal scores in male rats treated with morphine at 10 mg/kg body weight than in female rats receiving the same dose (Craft et al., 1999). Along similar lines, male mice demonstrated a significantly greater sensitivity to naloxone-

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precipitated withdrawal, as evidenced by their having an almost fourfold lower median effective dose (ED_{50}) for withdrawal compared to female mice (Kest et al., 2001). In contrast, Cicero et al. (2002) reported that no sex differences were observed during naloxone-precipitated withdrawal following chronic morphine administration, although male rats exhibited a more severe spontaneous withdrawal syndrome than female rats following abrupt cessation of morphine administration. Although naloxone dose and morphine administration schedule differed across the three studies, Cicero et al. (2002) interpreted the absence of sex differences during naloxone-precipitated withdrawal between male and female rats at equivalent doses to mean that morphine produced equivalent levels of physical dependence in both male and female animals. The authors, however, also acknowledged their inability to offer a reasonable explanation of this observation. More recent studies in rodents suggest that both sex and opioid maintenance dose influence the nature of opioid dependence and withdrawal. In a study examining spontaneous opiate withdrawal in mice using low-dose and high-dose morphine administration paradigms, female mice demonstrated somatic withdrawal signs up to 24 h longer than that demonstrated by male animals. The same study showed that opiate withdrawal also varied as a function of the cumulative morphine dose administered, such that animals treated with high-dose morphine experienced more severe and longer-lasting withdrawal signs than those receiving a low cumulative dose (Papaleo and Contarino, 2006).

Accumulating evidence also suggests that there are sex differences in behavioral and subjective responses to opioid agonists and antagonists in humans not dependent on opioids. A retrospective analysis of data from six studies showed that healthy female volunteers experienced higher ratings of 'coasting (spaced out),' 'heavy or sluggish feeling' and 'dry mouth' in response to equivalent (10 mg/70 kg, intravenous) doses of morphine (Zacny, 2001). Moreover, healthy female volunteers not abusing drugs have been shown to experience significantly lower ratings of 'coasting (spaced out)', 'heavy or sluggish feeling' and 'dry mouth' in response to a painful stimulus during butorphanol administration compared to male volunteers (Zacny and Beckman, 2004). Meanwhile, in a study examining sex differences in pain and negative affect during psychological stress following administration of naltrexone (50 mg), naltrexone produced increases in cold-induced pain intensity and unpleasantness in women, but not men, following the stress-evoking discouragement task (Frew and Drummond, 2007).

Given that there are very limited data about differences in opiate withdrawal in the opioid-dependent population, this study retrospectively examined data from five naloxone discrimination studies (Oliveto et al., 1998, 2002, 2003a,b, 2004) in which opioid-maintained male and female volunteers were exposed to naloxone (0.15 mg/70 kg IM) and placebo in double-blind, counterbalanced procedures. The specific aim of this investigation was to examine whether sex and opioid maintenance dose differentially altered the response to naloxone compared to placebo in individuals on opioid maintenance. By examining how such factors might influence naloxone-induced responses, we hoped to gain a better understanding of individual characteristics related to physical dependence and withdrawal. This might provide useful information for improving relapse prevention in opioid-dependent humans.

2. Methods

2.1. Participants

Data from five human naloxone discrimination studies examining the effects of intramuscular naloxone and hydromorphone (Oliveto et al., 1998; $n=10$ [2 females, 8 males]; Study 1); intramuscular naloxone, butorphanol and nalbuphine (Oliveto et al., 2002; $n=19$ [7 females, 12 males]; Study 2); naloxone, clonidine and yohimbine (Oliveto et al., 2003a; $n=14$ [6 females, 8 males]; Study 3); naloxone and cycloserine (Oliveto et al., 2003b; $n=7$ [4 females, 3 males]; Study 4); and naloxone, isradipine and dextromethorphan (Oliveto et al., 2004; $n=17$ [7 females, 10 males]; Study 5) were used in the present report. Subjects for Study 1 and the first part of Study 2 were recruited via newspaper advertisements targeting opiate-dependent individuals currently not in treatment. The rest of the subjects (remainder in Study 2 and those from Studies 3 to 5) were recruited by flyers specifically targeting those on opioid maintenance in local opioid maintenance programs and by word of mouth. All gave written informed consent to participate in a study and were compensated monetarily for their participation. These studies had been approved by the Yale Human Investigations Committee and/or the West Haven VA CT Healthcare System Human Studies Subcommittee.

Data from subjects in each study were included in the analyses if the following conditions were met: 1) the subject had completed at least the first two study sessions (training phase) such that they had received naloxone during one and placebo during the other, respectively; and 2) if a subject participated in more than one study, only data from the first study was used for that subject (Oliveto et al., 1998, 2002, 2003a,b, 2004). Thus, data were available from 10 subjects in study 1 (Oliveto et al., 1998), 13 subjects from study 2 (Oliveto et al., 2002), 9 subjects from study 3 (Oliveto et al., 2003a), 2 subjects from study 4 (Oliveto et al., 2003b), and 13 subjects from study 5 (Oliveto et al., 2004). Overall, data from 47 subjects (15 female and 32 male, aged 24 to 51 years) were included in these analyses.

Each subject's eligibility was determined through a comprehensive evaluation that included complete physical, neurological, and psychiatric examinations; laboratory chemistry tests; and electrocardiogram. For all studies, subjects had to meet the following inclusion criteria: (1) opioid dependence, as evidenced by either opioid-positive urine plus signs of withdrawal on a Narcan challenge test (all subjects in study 1 and three subjects in study 2), or currently in a methadone maintenance program (10 subjects from study 2 and all subjects from studies 3–5) in good standing (i.e., compliant with program rules, including no illicit drug use); (2) no major cardiovascular, renal, endocrine, or hepatic disorder; (3) no current diagnosis of other drug or alcohol physical dependence (except nicotine); (4) no history of major psychiatric disorder (e.g., schizophrenia, bipolar disorder, major depression); (5) no pregnancy or plans to become pregnant, if female; (6) no present or recent use of over-the-counter or prescription psychoactive drug or drug that would have a significant interaction with the drugs to be tested; (7) negative urine toxicology for illicit drugs upon entering the study. Information regarding the phase of the menstrual cycle or the use of oral contraceptives was not recorded for the female participants.

All subjects in Study 1 and the first 3 subjects in Study 2 participated on an in-patient basis and provided confirmation of opioid dependence via urine toxicology screen and signs of withdrawal upon administration of naloxone in a Narcan challenge test (0.2 to 0.8 mg IM). They were subsequently stabilized on methadone, by a procedure described earlier (Oliveto et al., 1998, 2002). The remainder of the participants in Study 2 and all the patients in Studies 3 to 5 were already receiving stable methadone or *levo*-α-acetyl methadol (LAAM) maintenance doses between methadone dose equivalents of 25 to 120 mg/day at local programs and participated on an out-patient basis.

2.2. General procedure

All subjects participating in Study 1 and the first 3 subjects in Study 2 were admitted to the Treatment Research Unit at the Connecticut Mental Health Center, an inpatient facility for patients and research subjects with psychiatric and substance-abuse problems, once they met eligibility criteria. These subjects were stabilized on methadone (dose range: 25 to 45 mg/day) prior to the study proper and remained on the inpatient unit until the end of their participation (approximately 4–

6 weeks). The rest of the subjects in Study 2 as well as in Studies 3–5 were already on a stable dose of methadone (dose range: 45 to 120 mg/day) or LAAM (40 to 100 mg/ alternate day) through their attendance at a local opioid maintenance program and participated on an outpatient basis at the Outpatient Behavioral Pharmacology Unit at the West Haven VA Connecticut Healthcare System. Two participants in Study 2 and one participant in Study 3 had been maintained on LAAM. Urine toxicology screens for benzoylecgonine and drugs other then those experimentally administered were performed before admission and on a random basis during the study proper. Breathalyzer and urine pregnancy tests (when appropriate) were also given at the time of admission and at weekly intervals for those participants participating on an inpatient basis, and daily and weekly intervals for those participating on an outpatient basis, to detect the presence of alcohol and possible pregnancy, respectively.

2.3. Experimental design

Data were obtained from the training phase of naloxone discrimination studies (see Oliveto et al., 1998 to 2004 for detailed explanations), during which subjects were exposed to the training dose of naloxone (0.15 mg/70 kg IM) and placebo (5% dextrose and 0.9% NaCl in the ratio of 1:1) twice (once in Study 1) in an alternating order. Naloxone and placebo were administered under double-blind conditions and were counterbalanced across subjects. Subjects were never informed of the actual identities of the drugs, but were given a list of drugs that they might receive during the course of the Study.

2.4. Drugs

All subjects were maintained on their maintenance doses of methadone or LAAM for the duration of their participation. Two volunteers in Study 2 were on thrice-weekly LAAM doses of 75 mg and 45 mg respectively. One participant in Study 3 was maintained on a LAAM dose of 50/50/75 mg per Mondays/Wednesdays/Fridays. These doses of LAAM were converted to methadone dose equivalent of 62.5 mg, 37.5 mg and 48.6 mg respectively, according to the formula suggested by the manufacturer (Roxane Laboratories Manufacturing Insert). For those participating on an inpatient basis, 5 subjects received their full dose of methadone 2 h after each session was completed. The rest of the subjects received half their dose of methadone prior to each session and the other half after each session was completed. For those participating as outpatients, subjects were instructed to take their full dose of methadone prior to each session.

Naloxone (0.15 mg/70 kg) was typically diluted in 0.9% sodium chloride (NaCl) and placebo vehicle consisted of NaCl. Each study drug was injected intramuscularly into the upper arm. Injections were prepared by the pharmacy on site. In all studies, the training conditions were administered 1 h prior to the first post-drug assessment period conducted 20 min following the injection.

2.5. Experimental session

Sessions typically began between 8:30 a.m. and 10:30 a.m. and occurred at least 24 h apart on weekdays. The timing of experimental sessions remained consistent within participants, who typically remained in the laboratory for approximately 4.5 to 5 h. At the beginning of each session, baseline field sobriety tests (i.e., tests of balance, hand coordination and simple arithmetic), a breathalyzer test (Alco-Sensor IV Intoximeter, Saint Louis, MO, outpatient participants only), and a computerized version of the Digit Symbol Substitution Task (DSST) were completed. In addition, a urine sample for random drug testing was submitted for those participating on an outpatient basis.

A set of assessments were carried out prior to the test injection that consisted of baseline self-report questionnaires (see below). Participants in Studies 3–5 were then administered placebo appropriate with and according to the individual study protocols that they had consented to participate in. Subsequently, an intramuscular injection of either naloxone or placebo was administered into the muscle of the upper arm at a consistent time following the placebo (Studies 3–5) or baseline assessments (Studies 1–2). Subsequently, participants completed assessments at two time-points, at 20 min and 40 min (hereafter 20 min and 40 min, respectively), after the intramuscular injection. Each assessment period lasted approximately 10 min and consisted of discrimination tasks, self-report measures and the taking of vital signs (see below). Vital signs were taken again at 60 min post-injection and then participants were given food. Those participating on an outpatient basis were evaluated one half hour later for release from the laboratory by undergoing the sobriety tests, completing the DSST and a test of memory. Performance needed to be within normal baseline parameters before these participants were released.

Participants were instructed to abstain from caffeine and food for at least 4 h before each session and were required to smoke their last cigarette from their regular brand about 10 min before the baseline field sobriety tests. No smoking was permitted from this time until after the completion of the session. Otherwise, participants could maintain a regular pattern of smoking for the remainder of the time. No food or beverage, except water, was allowed during the experimental sessions.

2.6. Dependent measures

2.6.1. Self-report measures

These were administered prior to and following the injection and included the following: shortened version of the Addiction Research Center Inventory (ARCI), Adjective Rating Scale and Visual Analog Scales (VAS). The ARCI is a standardized, self-report questionnaire for assessing subjective effects of psychoactive drugs. It consisted of 49 true/false questions that were scored as five sub-scales: morphine–benzedrine group (MBG), a measure of "euphoria"; pentobarbital–chlorpromazine–alcohol group (PCAG), a measure of "sedation"; lysergic acid diethyl amide (LSD), a measure of "dysphoria"; and the benzedrine group (BG) and amphetamine (A) scales, which are sensitive to *d*-amphetamine-like effects (Jasinski, 1977; Martin et al., 1971).

The Adjective Ratings Scale (ARS) was used to detect subjectively experienced opiate agonist, antagonist or mixed (agonist–antagonist) effects. The ARS listed 32 adjectives that were rated on a five-point scale from 0 (not at all) to 4 (extremely). The items in the list were grouped into three sub-scales: (1) *Agonist Scale*, consisting of the terms carefree, coasting or spaced out, drive, dry mouth, drunken, energetic, flushing, good mood, heavy or sluggish feeling, nodding, pleasant sick, relaxed, skin itchy, sleepy, sweating, talkative, tingling, and turning of stomach; (2) *Antagonist Scale*, consisting of the terms agitated, chills, goose flesh, restless, runny nose, shaky, tired, and watery eyes; and (3) *Mixed-Action Agonist/Antagonist Scale*, consisting of the terms confused, depressed, floating, headache, lightheaded, and numb (Preston et al., 1987).

Visual Analog Scales (VAS) were also designed and used during each of the naloxone discrimination studies mentioned above (Oliveto et al., 1998, 2002, 2003a,b, 2004). They consisted of eight 100-point horizontal lines anchored with "not at all" on one end and "extremely" on the other. On these scales, subjects marked the part of the line that represented the extent to which they experienced "any" drug effect, drug-liking, "good" drug effects, "bad" drug effects, drug-induced high, and effects similar to each training condition (identified by drug letter code) and to neither drug condition ("N"). These last three ratings were then translated, based on the training condition associated with each letter code, into ratings of 'like naloxone,' 'like placebo,' and 'like novel' (Bickel et al., 1993).

No direct measures of "opioid withdrawal", per se, were employed.

2.6.2. Physiological measures

Heart rate and blood pressure were taken at baseline and at 20 min and 40 min post-injection. These were measured with an automated

blood pressure cuff through a Dinamap Critikon 1846SX Vital Signs monitor (Shelton, CT). Pupil diameter was not measured.

2.7. Data analysis

Demographic and other substance-use characteristics available as continuous measures were compared between male and female subjects using *t*-tests. *t*-Tests were also used to examine baseline (prior to injection) differences on self-reported and physiological measures between men and women. Categorical variables were compared using the Chi-square test or the Fisher's exact test, if 2×2 cell counts were low. Two-sided table probabilities are reported.

Methadone dosage per kg body weight was categorized into high- or low-dose group by a median split for each sex (median=1.0476 for females and median=0.6707 for males) ensuring equal numbers in each drug condition for each sex. Scores on self-report and physiological measures are presented as change from baseline pre-drug values. We began by examining whether the residuals for all the dependent measures demonstrated a normal distribution. Data from only the VAS Any drug effects and VAS Bad drug effects sub-scales were found to violate this assumption of normality. Hence, log transformations of these data were carried out so that analyses using general linear models (GLM) could be performed.

Repeated measures analyses of variance (ANOVA) with methadone maintenance dose (high vs. low), sex (female vs. male), drug (naloxone vs. placebo) and post-drug time-point (20 min post-drug vs. 40 min post-drug) were performed for all dependent measures using SAS Proc MIXED. The mixed procedure in SAS allows for the generalization of the standard linear model (GLM) by allowing for the data to exhibit correlation and non-constant variability. The covariance structure among the between-subject factors was set as unstructured. Denominator degrees of freedom were computed in SAS Proc MIXED in the manner described by Schluchter and Elashoff (1990). All tests performed were two-tailed and the level of α (to infer statistical significance) was set at 0.05.

If the type 3 test for the four-way interaction 'time×drug×methadone dose per kg×sex' was found significant, planned post-hoc tests were carried out to examine differences within sex between the low-dose and high-dose methadone conditions, and whether these differences were significant at the 20 min or the 40 min time-points. Whether differences existed between male or female subjects in a particular dose group at a particular time-point was also tested. For the significant four-way effects, approximate *t*-tests of least square means were performed to evaluate the significance and location of differences between drug conditions, dosages, times, and sexes. If the four-way interaction was found non-significant, we tested the significance of the three-way interaction without sex; that is: time×drug×methadone dose per kg.

We also carried out additional analyses on measures showing significant four-way or three-way interactions to check whether certain peculiarities in our data might be responsible for some of the results that we observed. Specifically:

2.7.1.

To insure that any significant effects were not caused solely by the participants on LAAM, measures were reanalyzed without the three participants on LAAM.

2.7.2.

In order to test whether or not receiving less than the full opioid maintenance dose before the session had significant effect on the overall level of response, an additional variable was created wherein the first 12 participants receiving less than their full dose of methadone prior to each session were coded as 0, while all other participants were coded as 1. This variable was added as a covariate in the analyses.

2.7.3.

To ascertain whether timing of naloxone injection may have impacted results, the time to naloxone administration since taking the methadone was added as a covariate in all the significant analyses described above. It was seen that participants could be divided into two main groups, those who had received their last methadone dose many hours prior to naloxone administration and those who had taken their methadone a short time before the experimental sessions.

2.7.4.

In order to determine if sex differences were influenced by differences in the median split by sex for methadone dose per kg, the significant four-way and three-way models were re-fit with the grand-median split for the methadone dose (mg per kg) for the entire sample.

3. Results

3.1. Subject characteristics

Subject characteristics are shown in Table 1. Participants generally did not differ in subject characteristics, except that males reported a significantly longer duration of opiate use compared to females. In addition, although no significant sex differences in mean body weight were found, females were maintained on a significantly higher mean methadone dose per kilogram body weight compared to males (Table 1). The mean methadone doses (in mg/kg) between males and females in either the low- or the high-dose groups were significantly different (Table 1). In addition, the mean methadone dose for males in the high-dose group was significantly higher than

Table 1
Demographic and baseline substance-use characteristics of the study subjects

	Males (N=32)	Females (N=15)	Test statistic	Significance (p=)
Age in yrs (±SD)	37.5 (±7.4)	37.7 (±4.8)	t=−0.11, df=40.2	0.911
Weight in kg (±SD)	85.2 (±15.2)	77.3 (±28.3)	t=1.02, df=17.9	0.321
Education in yrs (±SD)	12.2 (±2.2)	12.1 (±2.6)	t=0.13, df=23.8	0.896
Race				
White (%)	20 (62.5)	11 (73.4)	χ^2=0.84	0.66
African American (%)	8 (25)	2 (13.3)		
Hispanic (%)	4 (12.5)	2 (13.3)		
Methadone dose (±SD)				
Total (mg)	60.0 (±27.3)	73.9 (±25.3)	t=−1.66, df=44	0.10
mg/kg body weight	0.72 (±0.34)	1.01 (±0.39)	t=−2.60, df=44	0.01
Low-dose group	0.44 (±0.14)	0.67 (±0.18)	t=−6.57, df=86	<0.0001
High-dose group	0.98 (±0.23)	1.31 (±0.23)	t=−6.57, df=94	<0.0001
Opioid use				
Years use (±SD)	11.97 (±7.8)	6.16 (±2.9)	t=3.68, df=43.9	0.008
Method				
Intravenous (%)	20 (62.5)	10 (66.7)	χ^2=0.66	0.72
Nasal inhalation (%)	11 (34.4)	4 (26.7)		
Other drugs used				
Alcohol (%)	22 (68.8)	9 (64.3)	χ^2=0.09	0.77
Cocaine (%)	30 (93.75)	15 (100)	χ^2=0.98	0.32
Cannabis (%)	22 (68.75)	7 (46.7)	χ^2=2.11	0.15
Hallucinogens (%)	10 (31.25)	1 (6.7)	χ^2=3.44	0.06
Amphetamine (%)	4 (12.5)	1 (6.7)	χ^2=0.37	0.55
Benzodiazepines (%)	14 (43.75)	6 (40)	χ^2=0.06	0.81
Nicotine (%)	30 (93.75)	15 (100)	χ^2=0.98	0.32

Numbers represent either Mean (±SD) values or (percentage) as appropriate.
SD: Standard deviation.

the mean dose for females in the low-dose group (t=6.45, df=90, p<0.0001).

3.2. Baseline ratings: self-reported and physiological measures

There were no significant differences between males and females on any of the baseline self-reported or physiologic measures obtained prior to drug administration during the experimental sessions (data not shown).

3.3. Sex differences (four-way interactions)

Measures with a significant four-way interaction are shown in Fig. 1. Significant time×drug condition×methadone dosage per

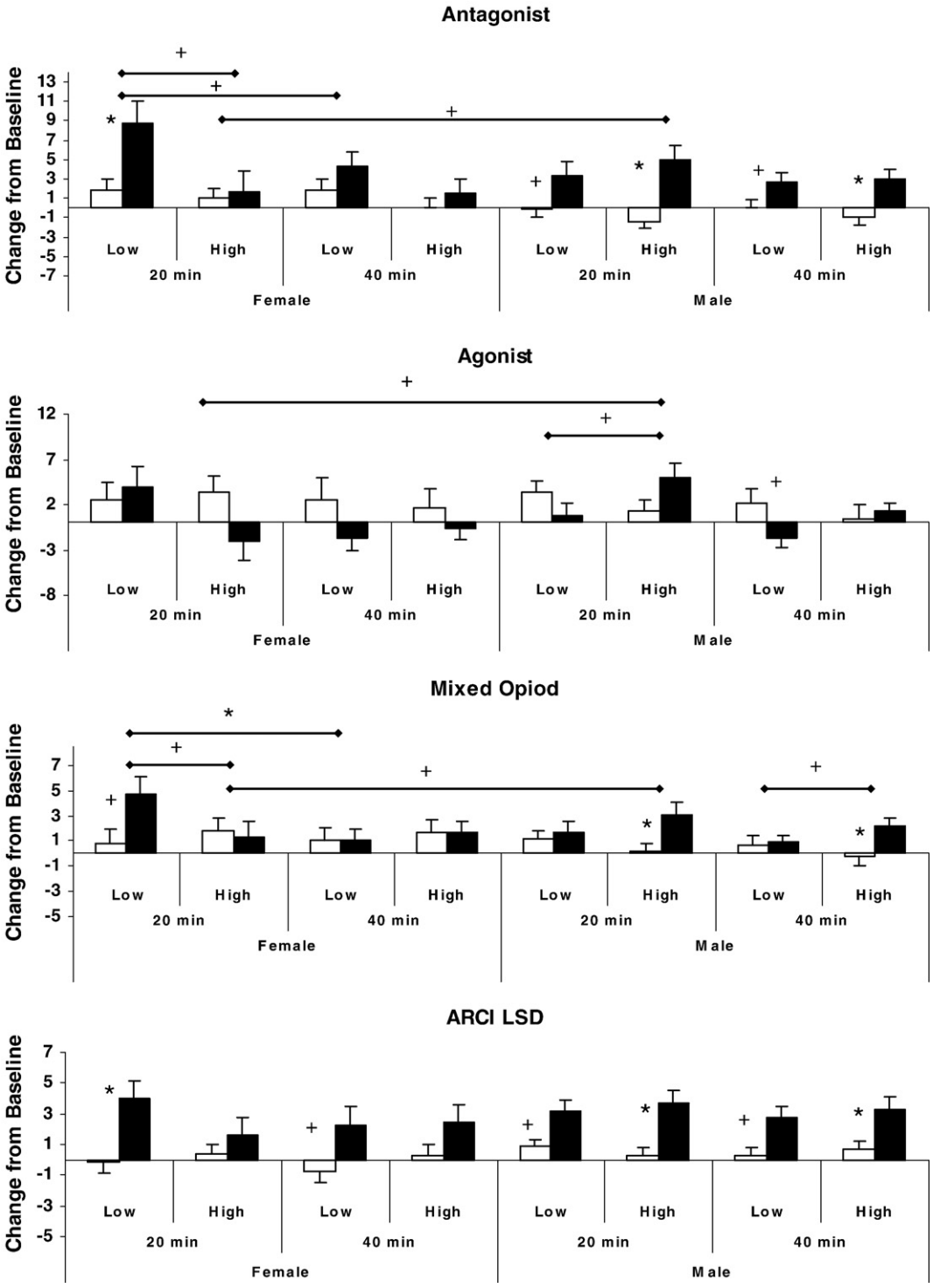

Fig. 1. A, B, C, D: Effects of 0.15 mg/70 kg of naloxone (filled bars) and placebo (open bars) on self-reported ratings on the opioid Antagonist (first graph), opioid Agonist (second graph), Mixed action opioid (third graph) sub-scales of the Adjective Ratings Scale and the LSD sub-scale (fourth graph) of the Addiction Research Center Inventory in females (left half of each graph) and males (right half) maintained on high- or low-dose methadone. Ordinate: mean change from pre-drug score. Abscissa: 20 min and 40 min post-drug time-points for the low- and high-dose methadone groups. Each bar represents the mean and standard error, +significant difference at $p \leq 0.05$, and *significant difference at $p \leq 0.005$.

kg × sex interactions were found for ratings for the opioid Antagonist sub-scale [$F(1,43)=4.83$, $p=0.033$], the opioid Agonist sub-scale [$F(1,43)=4.72$, $p=0.035$], and the Mixed-Action opioid agonist/antagonist sub-scale [$F(1,43)=6.13$, $p=0.017$] of the ARS, and the LSD sub-scale of the ARCI [$F(1,43)=4.02$, $p=0.050$].

3.3.1. Antagonist sub-scale ARS

Post-hoc hypothesis tests showed that females on low-dose methadone showed a significant naloxone-induced increase in the Antagonist sub-scale scores compared to placebo at 20 min post-injection ($t=-3.26$, $df=43$, $p=0.002$; see Fig. 1, top panel, left). Females on high-dose methadone did not experience a significant change in scores ($t=-0.38$, $df=43$, $p=0.70$). At the 40 min time-point, no differences in scores occurred between naloxone and placebo in either females on low-dose methadone ($t=-1.45$, $df=43$, $p=0.16$) or those on high-dose methadone ($t=-0.96$, $df=43$, $p=0.34$). The naloxone-placebo difference in scores on this sub-scale at the 20 min time-point was significantly greater than at the 40 min time-point for females on low-dose methadone ($t=-2.42$, $df=43$, $p=0.02$), but not on high-dose methadone ($t=0.44$, $df=43$, $p=0.66$). Female subjects on low-dose methadone also had a significantly greater naloxone-placebo difference in scores at the 20 min time-point compared to females on high-dose methadone ($t=-2.12$, $df=43$, $p=0.04$).

In male participants, naloxone produced a significantly greater change in Antagonist ARS scores compared to placebo at the 20 min assessment both in those on low-dose ($t=-2.52$, $df=43$, $p=0.016$) and high-dose methadone ($t=-4.54$, $df=43$, $p<0.001$; see Fig. 1, top panel, right). Similarly, significant naloxone-induced increases in Antagonist ARS scores compared to placebo at the 40 min time-point occurred in both those on low-dose ($t=-2.36$, $df=43$, $p=0.02$) and high-dose ($t=-3.55$, $df=43$, $p=0.001$) methadone. No significant differences in naloxone-placebo changes in scores between the 20 min compared to the 40 min time-point were seen in either males on low-dose ($t=-0.72$, $df=434$, $p=0.47$) or high-dose methadone ($t=-1.96$, $df=43$, $p=0.057$).

Finally, at the 20 min time-point, the naloxone-placebo change scores were significantly less in female subjects on high-dose methadone than male subjects on high-dose methadone ($t=2.31$, $df=43$, $p=0.03$).

3.3.2. Agonist sub-scale ARS

In females, no significant naloxone-placebo differences in Agonist sub-scale ratings occurred at either time-point in either methadone dose group.

In males, Agonist sub-scale ratings showed no significant naloxone-placebo differences at the 20 min time-point either in the low-dose ($t=1.40$, $df=43$, $p=0.17$) or the high-dose ($t=-1.91$, $df=43$, $p=0.06$) methadone groups (see Fig. 1, second panel, right). There was, however, a significant naloxone-induced decrease at the 40 min time period in males on low-dose methadone ($t=2.01$, $df=43$, $p=0.05$), but not high-dose methadone ($t=-0.49$, $df=43$, $p=0.63$). In addition, males on low-dose methadone had a significantly greater decrease in Agonist ARS scores at the 20 min time-point compared to males on high-dose methadone ($t=2.34$, $df=43$, $p=0.02$). These differences between the low-dose and high-dose groups were not observed at the 40 min time-point ($t=1.76$, $df=43$, $p=0.08$).

At the 20 min time-point, female subjects on high-dose methadone showed a significantly greater decrease in scores in the naloxone condition compared to placebo than male subjects on high-dose methadone ($t=2.68$, $df=43$, $p=0.01$).

3.3.3. Mixed-Action opioid sub-scale ARS

In females on low-dose methadone, post-hoc hypothesis tests showed a significant naloxone-induced increase in Mixed-Action opioid sub-scale scores at the 20 min time-point ($t=-2.58$, $df=43$, $p=0.01$) compared to placebo, but no difference between naloxone and placebo at 40 min post-injection ($t=0.0$, $df=43$, $p=1.0$; see Fig. 1, third panel, left). In contrast, no significant differences between naloxone and placebo occurred in females on high-dose methadone at either the 20 min ($t=0.45$, $df=43$, $p=0.66$) or the 40 min ($t=-0.11$, $df=43$, $p=0.91$) time-points. Females on low-dose methadone had significantly greater naloxone-placebo differences in scores at 20 min post-injection compared to 40 min ($t=-3.35$, $df=43$, $p=0.002$), as well as significantly greater naloxone-placebo change scores compared to the high-dose methadone group at the 20 min post-injection time-point ($t=-2.19$, $df=43$, $p=0.034$).

Male subjects on high-dose methadone experienced significantly greater naloxone-induced increases in Mixed-Action sub-scale scores compared to placebo at both the 20 min ($t=-3.03$, $df=43$, $p=0.004$) and the 40 min ($t=-3.09$, $df=43$, $p=0.004$) time-points (see Fig. 1, third panel, right). In contrast, males on low-dose methadone did not show any significant naloxone-induced increases in scores compared to placebo at either time-point.

At the 20 min time-point, male subjects on high-dose methadone showed a significantly greater change in scores in the naloxone condition compared to placebo than female subjects on high-dose methadone ($t=2.11$, $df=43$, $p=0.04$). Females on low-dose methadone showed a trend toward higher scores following naloxone compared to placebo than males on low-dose methadone ($t=-1.84$, $df=43$, $p=0.07$).

3.3.4. LSD sub-scale ARCI

Females on low-dose, but not high-dose, methadone showed a significant naloxone-induced increase in the LSD sub-scale scores compared to placebo at 20 min post-injection ($t=-3.41$, $df=43$, $p=0.001$ vs. $t=-1.10$, $df=43$, $p=0.28$; see Fig. 1, last panel, left). At the 40 min time-point, females on low-dose methadone ($t=-2.34$, $df=43$, $p=0.02$), but not high-dose methadone ($t=-1.88$, $df=43$, $p=0.07$), showed a significant naloxone-induced increase in LSD sub-scale scores.

In male participants, naloxone produced significantly greater increases in scores compared to placebo at the 20 min assessment both in those on low-dose ($t=-2.80$, $df=43$, $p=0.008$) and on high-dose ($t=-4.21$, $df=43$, $p<0.001$; see Fig. 1, last panel, right) methadone. Similarly, significant naloxone-induced increases in ARCI–LSD scores compared to placebo at the 40 min time-point occurred in both those on low-dose ($t=-2.87$, $df=43$, $p=0.006$) and high-dose ($t=-3.09$, $df=43$, $p=0.004$) methadone.

There were no significant differences in naloxone-placebo change scores between females and males on either low-dose methadone ($t=-1.30$, $df=43$, $p=0.20$) or high-dose methadone ($t=1.53$, $df=43$, $p=0.13$) at the 20 min time-point.

3.4. Differences that were not sex-dependent (three-way interactions)

Significant time × drug condition × methadone dosage per kg interactions were found for VAS Any drug effects [$F(1,43)=5.64$, $p=0.02$] and VAS Bad drug effects [$F(1, 43)=4.43$, $p=0.04$]. These data are shown in Fig. 2.

3.4.1. VAS Any drug effects

At the 20 min post-injection period, hypothesis tests showed significant naloxone-induced increases in the VAS Any drug effects scores compared to placebo for those on both low-dose ($t=-4.09$, $df=43$, $p<0.001$) and high-dose ($t=-2.77$, $df=43$, $p=0.008$) methadone (see Fig. 2, top panel). At the 40 min time-point, there was a significant naloxone-induced increase in scores for those on high-dose ($t=-3.54$, $df=43$, $p<0.001$), but not low-dose ($t=-1.26$, $df=43$, $p=0.22$) methadone. The naloxone-placebo differences on the VAS Any drug effects scores were significantly greater at the 20 min time-point compared to the 40 min time-point only for those on low-dose methadone ($t=-2.42$, $df=43$, $p=0.02$).

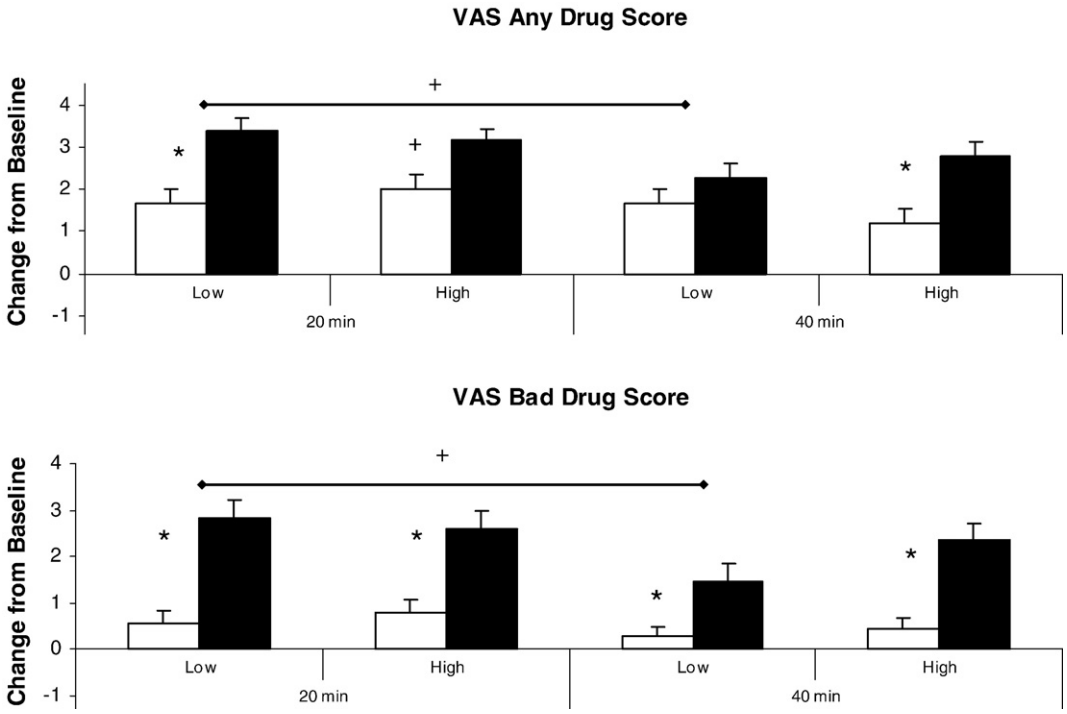

Fig. 2. A, B: Effects of 0.15 mg/70 kg of naloxone (filled bars) and placebo (open bars) on self-reported ratings on the Any Drug Effects (first graph) and the Bad Drug Effects (second graph) sub-scales of the Visual Analogue Scales on subjects maintained on high- or low-dose methadone. Ordinate: mean log-transformed change from pre-drug score. Abscissa: 20 min and 40 min post-drug time-points for the low- and high-dose methadone groups. Each bar represents the mean and standard error, +significant difference at $p \leq 0.05$, and *significant difference at $p \leq 0.005$.

3.4.2. VAS Bad drug effects

Hypothesis tests showed that naloxone produced significantly greater increases in VAS Bad drug effects scores compared to placebo both at the 20 min and 40 min time-points, and for both those on low-dose ($t=-5.21$, $df=43$, $p<0.001$ at 20 min, and $t=-3.05$, $df=43$, $p=0.004$ at 40 min, respectively) and those on high-dose ($t=-4.21$, $df=43$, $p<0.001$ at 20 min, and $t=-4.94$, $df=43$, $p<0.001$ at 40 min) methadone (see Fig. 2, bottom panel). Those on low-dose methadone also experienced a significantly greater naloxone-placebo difference in scores on VAS Bad drug effects scores at 20 min compared to 40 min ($t=-2.69$, $df=43$, $p=0.01$).

3.5. Physiological measures

No statistically significant differences between any of the comparison groups were found for any of the physiological measures.

3.6. Additional analyses

3.6.1.

After excluding the three participants on LAAM, all three-way results and all major findings of the four-way interactions remained unchanged. Only two of the more minor findings in the four-way results changed from being significant to that approaching significance. For instance, instead of a significant difference, a trend towards a significantly greater difference in the naloxone-placebo changes in scores on the ARS Antagonist sub-scale at the 20 min time-point compared to the 40 min time-point for females on low-dose methadone was observed ($t=-1.89$, $df=40$, $p=0.07$ from $t=-2.42$, $df=43$, $p=0.02$). In addition, females on low-dose methadone now showed a trend towards significantly greater naloxone-placebo difference scores at 20 min post-injection compared to the high-dose methadone group ($t=-1.74$, $df=40$, $p=0.09$ from $t=-2.19$, $df=43$, $p=0.034$) on the ARS Mixed-Action sub-scale.

3.6.2.

Addition of the full-dose variable (described above) as a covariate to the significant four-way and three-way models resulted in no significant changes. For the three-way models, this variable was added to test the four-way effect of dosage timing × time × drug condition × methadone dosage per kg interactions. There were no significant changes for either the VAS Any drug effects or the VAS Bad drug effects. There were not enough degrees of freedom to test the five way interaction of dosage timing × time × drug condition × methadone dosage per kg × sex interactions for Antagonist, Agonist, and Mixed-Action of the ARS and LSD of the ARCI.

3.6.3.

The time between the last methadone dose and naloxone administration was not found to influence the significant four-way or three-way interactions in any manner when it was added as a covariate in the analyses.

3.6.4.

Reanalysis of the data using a grand-median split for all the participants, irrespective of sex, also did not alter the results in any manner.

4. Discussion

There were three major observations from our analyses. First, female subjects, especially those on low-dose methadone, showed a large naloxone-induced increase on the Antagonist and Mixed-Action agonist/antagonist sub-scales of the ARS and the LSD sub-scale of the ARCI predominantly at the 20 min post-naloxone time-point. Second, male subjects on high-dose methadone showed more modest, but sustained, naloxone-induced increases on the Antagonist and Mixed-Action agonist/antagonist sub-scales of the ARS at both the 20 min and 40 min time-points. Finally, regardless of sex, those on low-dose methadone showed greater, but short-lived, while those on high-dose

methadone showed more modest, but longer lasting, naloxone-induced increases on the VAS Any drug effects and Bad drug effects ratings post-naloxone administration.

4.1. Participant characteristics

The duration of opiate use for female participants was significantly less than for male participants, which is consistent with reports that female drug users reportedly have a more rapid progression of drug use, as well as earlier contact with treatment compared to males (Peles and Adelson, 2006; Westermeyer and Boedicker, 2000). Female participants were also maintained on a higher methadone dose per kg of body weight compared to males. Data from the very few human studies of opiate abusers have generally reported no significant differences in either total amounts of opiate used (Hser et al., 1987) or in methadone maintenance dose (Peles and Adelson, 2006). Subgroup means of the total methadone dose between males and females were not significantly different in our study as well (Table 1). However, there was a significant difference in mean methadone doses between male and female volunteers when body weight was taken into account (i.e. in mg/kg body weight). The reason for this is unclear, but is consistent with the data from preclinical studies in which female rodents were observed to consume greater amounts of opiates compared to their male counterparts (e.g. Carroll et al., 2001). Nevertheless, this result could also be merely due to the relatively small, unbalanced sample of female and male participants.

4.2. Self-reported effects

Our discrimination studies did not use any direct measures of opiate withdrawal or measure pupil diameters. However, significant differences on the ratings of the opioid Antagonist sub-scale of the ARS, LSD sub-scale of the ARCI, and Any and Bad drug effects sub-scales of the VAS between the naloxone and placebo conditions is consistent with prior studies examining the discriminative stimulus effects of naloxone in opioid-dependent individuals (e.g., Oliveto et al., 1998, 2002; Preston et al., 1987). These results most likely reflect the expression of naloxone-precipitated opioid withdrawal. That female participants on low-dose methadone reported the greatest naloxone-induced changes on several self-reported measures at the first post-drug assessment time-point suggests that female volunteers, especially those on low-dose methadone, were perhaps more sensitive to the opioid withdrawal-like effects of naloxone during opiate maintenance. The reasons for this sex difference remain to be elucidated, but do not appear to be due to differences in maintenance dose between males and females for several reasons. For instance, females on low-dose methadone were maintained on a higher milligram per kilogram average dose of methadone than males on low-dose methadone. Thus, any greater naloxone-induced changes in scores that occurred in females did so under more conservative conditions. Additionally, when the data were reanalyzed using a grand-median split across both males and females, these effects were similar to the results from the analysis with the median split by sex (data not shown).

Naloxone is rapidly metabolized in humans by glucuronidation in the liver (Handal et al., 1983) and both methadone and LAAM are metabolized predominantly by cytochrome P450 3A4 group of enzymes (Oda et al., 2001). There is no evidence in the literature to suggest that metabolic differences between men and women for these drugs are responsible for the differential responses observed in this study. On a different note, a study by Zubieta et al. (2002) showed that females, during the follicular phase of their menstrual cycles, and males differed significantly in both the magnitude and direction of the responses of distinct brain nuclei at matched levels of pain intensity. Using positron emission tomography and a mu (μ)-opioid receptor selective radiotracer in subjects within a narrow age range and controlling for phase of menstrual cycle, this study found that female subjects demonstrated higher μ-opioid receptor concentrations in the amygdala region compared to male subjects. This higher μ-opioid receptor density in females is likely to result in a greater sensitivity to the effects of exogenously administered μ-opioid agonists or antagonists. The findings from our study provide support for the imaging observations by Zubieta et al. (2002) because study subjects who were female, especially those on low-dose methadone, were found to be more sensitive to the effects of naloxone administration compared to other females on high-dose methadone or to male subjects. Hence, individual differences in brain physiology and receptor density are likely explanations for the observed differences in responses observed, instead of differences in opiate metabolism.

Animal models also demonstrate that sudden blockade of the μ-opiate receptors initiates a cascade of events including activation of the brain noradrenergic system starting in the locus coeruleus (Espejo et al., 2001). This eventually results in norepinephrine-mediated activation of the brain stress systems from the release of corticotrophin releasing factor (CRF) by the hypothalamus and the amygdala (Camí and Farré, 2003). A recent study also showed that CRF was between 10 and 30 times more potent in activating the locus coeruleus in female rats compared to male rats (Curtis et al., 2006). Hence, it is more likely that intrinsic, sex-related differences in the sensitivity and responses of the human brain to the effects of opioids explain the sex- and dose-related differences observed in our subjects. Similar hypotheses have been proposed with regards to sex-related differences in opioid-mediated nociception and analgesia in humans, which have been reviewed by Kest et al. (2000). Consistent with previous findings of heightened sensitivity to pain in women, Frew and Drummond (2007) found that women were less tolerant of cold-induced pain than men and more sensitive to the μ-opiate receptor blocking effects of naloxone, as evidenced by greater increases in the intensity and unpleasantness of pain following discouragement. Sex differences in the brain's experience of negative affect in humans were offered as an explanation of this observation, although the exact mechanisms remain to be elucidated.

In addition to the significant sex-related differences, the three-way interactions observed on the two VAS ratings suggest that the effect of an equivalent dose of naloxone also varies depending upon the amount of methadone present. Naloxone produced a more intense, but short-lived, effect in those on low-dose methadone, as evidenced by greater changes on the VAS Any and Bad drug effects at the 20 min time-point. However, the response to naloxone in those on high-dose methadone was longer lasting but less intense, and not always significantly different from placebo. The impact of opioid dose on naloxone activity at the opiate receptors is yet to be explored. However, findings from rodent studies show that a more severe spontaneous withdrawal syndrome was observed in rats in the high-dose morphine group compared to those who had received a lower morphine dose (Papaleo and Contarino, 2006). In addition, Camarasa et al. (2007) noticed a two-phase course of withdrawal in a group of 10 methadone-substituted patients during naltrexone administration. The first phase was attributed to the antagonistic effects of naltrexone, while the second phase was hypothesized to be from a falling plasma level of methadone. Thus, there is accumulating evidence that the maintenance dose of an opiate agonist might influence the subjective experience of the resulting withdrawal from that dose. Future research is necessary to explore the effect of the opiate maintenance dose on differences in spontaneous or naloxone-precipitated withdrawal in humans.

4.3. Comment

While the exact significance of our observations is unclear at this time, the findings suggest that naloxone-precipitated withdrawal may be influenced by sex and opioid maintenance dose in humans. It is possible that certain characteristics unique to our retrospective analyses

of pooled data could possibly have affected the results observed. However, we were able to rule out several factors as potential confounds by additional analyses, including whether or not the full dose of methadone was ingested prior to the session, whether or not data from LAAM-maintained participants were included in the analyses, the time following methadone intake when the naloxone was injected and the opioid maintenance dose median split employed in the analysis.

Nevertheless, several other limitations remain. For instance, the timing of menstruation in female participants was not recorded. Controlling for stage of menstrual cycle might be important given that female animals have been found to be more sensitive to the rewarding effects of drugs in the luteal phase of the cycle in animal studies (Carroll et al., 2004; Lynch et al., 2002). However, data from studies in humans has been equivocal about any effect of the menstrual cycle phase (Frew and Drummond, 2007). Be that as it may, menstrual cycles tend to be suppressed or are typically irregular in treatment seeking opioid-dependent females (Santen et al., 1975), as well as in those who are already maintained on methadone (Schmittner et al., 2005). Nevertheless, the differential response to naloxone in females based on methadone dose may reflect relative differences in the ability of agonist maintenance doses to suppress menstrual cycles. More research is necessary to clarify the role of differences in the menstrual cycle stage with regards to the response to naloxone in this population.

There are other limitations related to procedures or differences across studies that could not be accounted for with the small sample sizes. For instance, studies were conducted in both outpatient or inpatient settings, and the timing of methadone ingestion relative to an experimental session varied in the outpatient studies. There were also fewer female participants compared to males in this analysis, and any difference in results from having balanced numbers of female and male participants will remain unknown. Next, only a single dose of naloxone was used for investigation and it might have been informative to examine the effect of different doses (in mg/kg) of naloxone. More importantly, these data are from human studies of naloxone-precipitated opiate withdrawal. Data from a study on rats suggests that withdrawal precipitated by an opiate antagonist might be different from spontaneous opiate withdrawal (Cicero et al., 2002). However, while it might be acceptable to study the withdrawal from a short-acting antagonist, like naloxone, ethical issues might arise while observing spontaneously occurring withdrawal and withholding opiate agonist treatment in human subjects. Finally, we were also unable to control for the longer duration of opiate abuse at baseline observed among male subjects compared to females.

Despite these limitations, there are several strengths and some interesting hypotheses that can emerge from this investigation. First, naloxone was administered to all subjects in a double-blind, placebo-controlled, cross-over design, allowing for comparison of sex differences in naloxone-precipitated withdrawal under similar conditions. Next, we attempted to control for differences in mean methadone doses by dividing the two groups into low- and high-dose methadone, in mg/kg body weight, based on the median split for each sex. In addition, even though preliminary in nature, this study is, to our knowledge, among the first to examine factors influencing response to an opioid antagonist in opiate-dependent individuals with substance-abuse histories, rather than healthy subjects with no history of substance abuse. The findings suggest that females on lower methadone maintenance doses might be particularly sensitive to the unpleasant and dysphoric effects of naloxone-precipitated opiate withdrawal compared to the other groups. Further research is necessary to understand fully the implications of these sex and methadone dose differences in response to naloxone in opioid-maintained humans.

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References

Alexander BK, Coambs RB, Hadaway PF. The effect of housing and gender on morphine self-administration in rats. Psychopharmacology 1978;58:175–9.
Barrett AC. Low efficacy opioids: implications for sex differences in opioid antinociception. Exp Clin Psychopharmacol 2006;14:1–11.
Bickel WK, Oliveto AH, Kamien JB, Higgins ST, Hughes JR. A novel-response procedure enhances the specificity and sensitivity of a triazolam discrimination in humans. J Pharmacol Exp Ther 1993;264:360–7.
Camarasa X, Khazaal Y, Besson J, Zullino DF. Naltrexone-assisted rapid methadone discontinuation: a pilot study. Eur Addict Res 2007;13:20–4.
Camí J, Farré M. Drug addiction. N Engl J Med 2003;349:975–86.
Carroll ME, Campbell UC, Heideman P. Ketoconazole suppresses food restriction-induced increases in heroin self-administration in rats: sex differences. Exp Clin Psychopharmacol 2001;9:307–16.
Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK. Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. Psychopharmacology 2002;161:304–13.
Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. Trends Pharmacol Sci 2004;25:273–9.
Cicero TJ, Nock B, Meyer ER. Gender-linked differences in the expression of physical dependence in the rat. Pharmacol Biochem Behav 2002;72:691–7.
Craft RM, Stratmann JA, Bartok RE, Walpole TI, King SJ. Sex differences in development of morphine tolerance and dependence in the rat. Psychopharmacology 1999;143:1–7.
Craft RM, Clark JL, Hart SP, Pinckney MK. Sex differences in locomotor effects of morphine in the rat. Pharmacol Biochem Behav 2006;85:850–8.
Curtis AL, Bethea T, Valentino RJ. Sexually dimorphic responses of the brain norepinephrine system to stress and corticotrophin-releasing factor. Neuropsychopharmacology 2006;31:544–54.
Espejo EF, Serrano MI, Caille S, Stinus L. Behavioral expression of opiate withdrawal is altered after prefrontocortical dopamine depletion in rats: monoaminergic correlates. Neuropsychopharmacology 2001;25:204–12.
Fillmore KM, Golding JM, Leino EV, et al. Patterns and trends in women's and men's drinking. In: Wilsnack RW, Wilsnack SC, editors. Gender and alcohol: individual and social perspectives. Rutgers Center of Alcohol Studies, New Brunswick, NJ; 1997. p. 21–48.
Frew AK, Drummond PD. Negative affect, pain and sex: the role of endogenous opioids. Pain 2007;132:S77–85.
Hadaway PF, Alexander BK, Coambs RB, Beyerstein B. The effect of housing and gender on preference for morphine-sucrose solutions in rats. Psychopharmacology 1979;66:87–91.
Handal KA, Schauben JL, Salamone FR. Naloxone. Ann Emerg Med 1983;12:438–45.
Hser YI, Anglin MD, Booth MW. Sex differences in addict careers. 3. Addiction. Am J Drug Alcohol Abuse 1987;3:231–51.
Hser YI, Hoffman V, Grella CE, Anglin MD. A 33-year follow-up of narcotics addicts. Arch Gen Psychiatry 2001;58:503–8.
Jackson A, Stephens D, Duka T. Gender differences in response to lorazepam in a human drug discrimination study. J Psychopharmacol 2005;19:614–9.
Jasinski DR. Assessment of the abuse potential of morphine-like drugs (methods used in man). In: Martin WR, editor. Drug Addiction I. New York, NY: Springer-Verlag; 1977. p. 197–258.
Kest B, Sarton E, Dahan A. Gender differences in opioid-mediated analgesia: animal and human studies. Anesthesiology 2000;93:539–47.
Kest B, Palmese CA, Hopkins E, Adler M, Juni A. Assessment of acute and chronic morphine dependence in male and female mice. Pharmacol Biochem Behav 2001;70:149–56.
Klein LC, Popke JE, Grunberg NE. Sex differences in effects of predictable and unpredictable footshock on fentanyl self-administration in rats. Exp Clin Psycopharmacol 1997;5:99–106.
Lancaster FE, Spiegel KS. Sex differences in pattern of drinking. Alcohol 1992;9:415–20.
Levine HJ, Reif S, Lee MT, Ritter GA, Horgan CM. Alcohol and drug services study (ADDS). The national treatment system: outpatient methadone facilities. Rockville, SAMHSA, Office of Applied Studies (OAS); 2004.
Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology 1999;144:77–82.
Lynch WJ, Roth ME, Carroll ME. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology 2002;164:121–37.
Martin WR, Sloan JW, Sapiro JD, Jasinski DR. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine and methylphenidate in man. Clin Pharmacol Ther 1971;12:245–58.
Oda Y, Kharasch ED. Metabolism of methadone and *levo*-α-acetylmethadol (LAAM) by human intestinal cytochrome P450 3A4: potential contribution of intestinal metabolism to presystemic clearance and bioactivation. J Pharmacol Exp Ther 2001;298:1021–31.
Oliveto AH, Rosen MI, Kosten TA, Hameedi FA, Woods SW, Kosten TR. Hydromorphone-naloxone combinations in opioid-dependent humans under a naloxone novel-response discrimination procedure. Exp Clin Psychopharmacol 1998;6:169–78.

Oliveto A, Sevarino K, McCance-Katz E, Feingold A. Butorphanol and nalbuphine in opioid-dependent humans under a naloxone discrimination procedure. Pharmacol Biochem Behav 2002;71:85–96.
Oliveto A, Sevarino K, McCance-Katz E, Benios T, Poling J, Feingold A. Clonidine and yohimbine in opioid-dependent humans responding under a naloxone novel-response discrimination procedure. Behav Pharmacol 2003a;14:97–109.
Oliveto A, Benios T, Gonsai K, Feingold A, Poling J, Kosten TR. D-cycloserine-Naloxone interactions in opioid-dependent humans under a novel-response naloxone discrimination procedure. Exp Clin Psychopharmacol 2003b;11:237–46.
Oliveto A, Poling J, Kosten TR, Gonsai K. Isradipine and dextromethorphan in methadone-maintained humans under a naloxone discrimination procedure. Eur J Pharmacol 2004;491:157–68.
Papaleo F, Contarino A. Gender- and morphine dose-linked expression of spontaneous somatic opiate withdrawal in mice. Behav Brain Res 2006;170:110–8.
Peles E, Adelson M. Gender differences and pregnant women in a methadone maintenance treatment (MMT) clinic. J Addict Dis 2006;25:39–45.
Perkins KA, Jacobs L, Sanders M, Caggiula AR. Sex differences in the subjective and reinforcing effects of cigarette nicotine dose. Psychopharmacology 2002;163:194–201.
Preston KL, Bigelow GE, Bickel WK, Liebson IA. Drug discrimination in human post-addicts: agonist-antagonist opioids. J Pharmacol Exper Ther 1987;243:1002–9.
Randall CK, Kraemer PJ, Bardo MT. Morphine-induced conditioned place preference in preweanling and adult rats. Pharmacol Biochem Behav 1998;60:217–22.
Santen FJ, Sofsky J, Bilic N, Lippert R. Mechanism of action of narcotics in the production of menstrual dysfunction in women. Fertility & Sterility 1975;26(6):538–48.
Singha AK, McCance-Katz EF, Petrakis I, Kosten TR, Oliveto A. Sex differences in self-reported and physiological response to oral cocaine and placebo in humans. Am J Drug Alcohol Abuse 2000;26:643–57.
Schluchter MD, Elashoff JT. Small-sample adjustments to tests with unbalanced repeated measures assuming several covariance structures. J Stat Comput Simul 1990;37:69–87.
Schmittner J, Schroeder JR, Epstein DH, Preston KL. Menstrual cycle length during methadone maintenance. Addiction 2005;100(6):829–36.
Westermeyer J, Boedicker AE. Course, severity, and treatment of substance abuse among women versus men. Am J Drug Alcohol Abuse 2000;26:523–35.
Zacny JP. Morphine responses in humans: a retrospective analysis of sex differences. Drug Alcohol Depend 2001;63:23–8.
Zacny JP, Beckman NJ. The effects of a cold-water stimulus on butorphanol effects in males and females. Pharmacol Biochem Behav 2004;78:653–9.
Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. μ-Opioid receptor-mediated antinociceptive responses differ in men and women. J Neurosci 2002;22:51005107.