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Isradipine and dextromethorphan in methadone-maintained humans under a naloxone discrimination procedure

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Abstract

In seven methadone-maintained human participants trained to distinguish between a low dose of naloxone (0.15 mg/70 kg, i.m.; i.e., Drug A) and placebo (i.e., Drug B) under an instructed novel-response drug discrimination procedure, the calcium channel blocker isradipine (0–10 mg/70 kg, p.o.; $N=7$) and the *N*-methyl-D-aspartic acid (NMDA) receptor antagonist dextromethorphan (0–60 mg/70 kg, p.o.; $N=6$) were tested each alone and in combination with the training dose of naloxone. Isradipine alone produced some naloxone- and novel-appropriate responding, minimal changes in self-reports and decreases in blood pressure. Dextromethorphan alone produced some novel-appropriate responding and minimal changes in self-reports and vital signs. When combined with naloxone, isradipine significantly attenuated naloxone-occasioned responding, without increasing novel-appropriate responding, and attenuated naloxone-induced increases in opioid receptor antagonist ratings and ratings measuring sedation. Dextromethorphan significantly attenuated naloxone-appropriate responding, increased novel-appropriate responding, and enhanced naloxone's effects on ratings of dysphoric effects. These results suggest that isradipine attenuates and dextromethorphan enhances some of the behavioral effects of naloxone in opioid-dependent humans.

Keywords: Opioid dependence; Opioid withdrawal; Naloxone; (Human); Isradipine; Dextromethorphan; Drug discrimination

1. Introduction

Opioid dependence is a severe public health problem (Zweben and Payte, 1990) and current efforts to taper individuals off opioid medications are limited by a high relapse rate (Broers et al., 2000; Gossop et al., 1989; Kleber et al., 1985; Rounsaville et al., 1985) and lack of efficacy in relieving subjective symptoms (Jasinski et al., 1985; Kosten et al., 1985; San et al., 1990). Thus, in order to improve upon treatments for opioid dependence, it is important to determine the degree to which pharmacological mechanisms found to be involved in the expression of opioid withdrawal in nonhumans are related to those in humans.

Although the mechanisms of opioid withdrawal are not well understood, there exists evidence to suggest that the expression of opioid withdrawal is mediated, in part, by μ-opioid and other neurotransmitter systems acting through the locus coeruleus (e.g., see Koob et al., 1992; Nestler, 1992; Redmond and Krystal, 1984). The locus coeruleus, the largest noradrenergic nucleus in the brain, also receives a major excitatory amino acid input from the nucleus paragigantocellularis (Akaoka and Aston-Jones, 1991; Ennis and Aston-Jones, 1988). Evidence suggests excitatory amino acids play a role in opiate dependence and withdrawal (e.g., Rasmussen and Aghajanian, 1989; Akaoka and Aston-Jones, 1991; Kogan and Aghajanian, 1995; Tokuyama et al., 2001). For instance, lesions of the paragigantocellularis-locus coeruleus pathway, or the application of excitatory amino acid glutamate receptor antagonists intracerebroventricularly or locally into the locus coeruleus, partially attenuate withdrawal activation of the locus coeruleus (Rasmussen and Aghajanian, 1989; Akaoka and Aston-Jones, 1991; Guyenet and Young, 1987).

One site where excitatory amino acid neurotransmission occurs is the *N*-methyl-D-aspartic acid (NMDA) receptor. This receptor is part of a receptor/ion channel complex with multiple regulatory sites, including the following: a L-glutamate recognition site for NMDA competitive receptor antagonists, ion channel recognition sites for noncompetitive NMDA receptor antagonists, a strychnine-insensitive glycine

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modulatory site, a polyamine site for NMDA noncompetitive receptor antagonists, and sites on cationic channels permeable to potassium, sodium and calcium (Mayer and Miller, 1990). NMDA receptor antagonists decrease the development of tolerance and physical dependence in opiate-treated animals (Trujillo and Akil, 1991; Marek et al., 1991; Tiseo and Inturrisi, 1993) as well as decrease the severity of naloxone-precipitated withdrawal in opiate dependent rats (Rasmussen et al., 1991). In addition, partial agonists or antagonists at the strychnine-insensitive glycine modulatory site have been shown to attenuate opioid receptor antagonist-precipitated withdrawal in nonhumans (e.g., Belozertseva et al., 2000; Bristow et al., 1997; Kosten et al., 1995; Popik et al., 1998). Activation of the NMDA receptor antagonist is also associated with increased intracellular calcium levels, with calcium channel blockers attenuating precipitated opiate withdrawal (Bongianni et al., 1986; Baeyens et al., 1987; Ramkumar and El-Fakahany, 1988; Silverstone et al., 1992).

A naloxone discrimination procedure in opioid-dependent humans has been used in our laboratory as a laboratory model of opioid withdrawal to examine whether drugs with pharmacological mechanisms thought to be involved in the expression of opiate withdrawal are similar to or alter the behavioral effects of naloxone (Oliveto et al., 1998, 2002, 2003a,b). Results showed that the opioid agonist hydromorphone produced novel-appropriate responding alone and blocked naloxone-occasioned responding and self-reported effects produced by naloxone in a dose-dependent manner, indicating a competitive interaction between these agents (Oliveto et al., 1998). Moreover, the naloxone stimulus partially generalized to the mixed-action opioids nalbuphine and butorphanol, which also produced novel-appropriate responding, indicating that the naloxone stimulus has pharmacological specificity (Oliveto et al., 2002). In addition, the naloxone stimulus partially generalized to the α_2-adrenoceptor antagonist yohimbine but not the α_2-adrenoceptor agonist clonidine, a finding consistent with the pharmacological profiles of these agents in opiate withdrawal. Clonidine also attenuated naloxone-occasioned responding and some self-reported effects produced by naloxone in a manner not dose-related while producing some novel-appropriate responding, illustrating a noncompetitive interaction between clonidine and naloxone (Oliveto et al., 2003a). Finally, the partial glycine agonist D-cycloserine produced minimal drug-appropriate responding and self-reported effects, and partially attenuated the discriminative stimulus effects and a few self-reported effects of naloxone (Oliveto et al., 2003b). These findings demonstrate the usefulness of this procedure as a model of opiate withdrawal for examining both opioid and nonopioid mechanisms underlying the expression of this phenomenon.

The present study examined the pharmacological mechanisms underlying the behavioral effects of naloxone in opioid-dependent humans responding under a naloxone discrimination procedure by examining the calcium channel blocker isradipine and the NMDA glutamate receptor antagonist dextromethorphan each alone and in combination with naloxone. Data in opioid-treated nonhumans have demonstrated that calcium channel blockers (e.g., (Alfaro et al., 1990; Kishioka et al., 1996; Krystal et al., 1996; Tokuyama et al., 1996) and NMDA glutamate receptor antagonists (e.g., Bristow et al., 1997; Farzin, 1999; Popik et al., 1998; Watanabe et al., 2002) attenuate naloxone- or naltrexone-precipitated opiate withdrawal. Thus, our hypothesis was that isradipine and dextromethorphan would produce minimal naloxone-appropriate responding and attenuate the discriminative stimulus and possibly self-reported effects of naloxone.

2. Materials and methods

2.1. Subjects

Seven female and ten male opioid-dependent volunteers (ages 24–51) were recruited from the APT Foundation's Methadone Maintenance Programs and the Opiate Treatment Program of the VA CT Healthcare System. Each subject gave written informed consent to participate in this study. Subjects each met the following inclusion criteria: (1) maintenance on a stable dose of methadone between 20 and 120 mg/day; (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; (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) good standing in an opioid agonist treatment program (i.e., compliant with program rules, including no illicit drug use); (8) negative urine toxicology for illicit drugs upon entering the study. Eligibility was ascertained through a comprehensive evaluation including complete physical, neurological, and clinical psychiatric examinations, routine laboratory studies, and electrocardiogram. Subjects were paid a base wage of US$6.00 per hour and awarded an additional US$9.00 per session depending on their discrimination performance (see Section 2.5). Subjects also accrued an additional US$200.00 for participating in the final study session. This protocol complied with the recommendations of the Declarations of Helsinki and Tokyo and was approved by the VA CT Healthcare System Human Subjects Subcommittee, the Yale University Human Investigations Committee, and the APT Foundation Committee Board.

Eleven subjects were Caucasian, five were African American and one was Hispanic. Subjects reported being in good standing in a current opioid agonist treatment program for a mean of 4.9 months (range: 1–15 months) and this was verified by research staff. Prior to treatment, subjects reported using opiates for a mean of 11.8 years (range: 3.5–30). Fourteen subjects were regular tobacco

smokers, reporting use of 16.1 cigarettes/day (range: 3.5–25). Although in good standing at their respective treatment program, subjects reported using the following at least once during the month prior to study entry: cocaine (5/17), alcohol (3/17), and marijuana (4/17). However, subjects submitted negative urine samples prior to study entry. Subjects weighed a mean of 86.9 kg (range: 56.4–152.7) at admission into the study.

2.2. Setting

This study was conducted at the Outpatient Behavioral Pharmacology Laboratory at the VA Connecticut Healthcare System, West Haven Campus. The laboratory consisted of a four-station experimental room with an adjacent lounge, where subjects could relax after the experimental portion of the session while waiting for drug effects to subside. A research nurse administered all medications and was present for the entire session. A physician was available during each session.

2.3. Experimental procedure

Subjects were trained to discriminate 0.15 mg/70 kg naloxone, injected intramuscularly (i.m.) into the upper arm, from placebo (vehicle, see Section 2.6) under an instructed novel-response (i.e., active dose, placebo dose, "novel") discrimination procedure (Bickel et al., 1993; Kamien et al., 1994; Oliveto et al., 1994, 1998). An initial session in which drug was not administered was conducted to familiarize subjects with the procedures. The study then proceeded in three phases:

2.3.1. Training (phase 1)

Subjects were exposed to naloxone (0.15 mg/70 kg, i.m.) and placebo twice each in a randomized order and *were informed* of the drug's letter code (e.g., Drug A or Drug B) at the time of drug administration. 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. Letter codes associated with the training drug stimuli were varied across subjects.

2.3.2. Tests-of-acquisition (phase 2)

To ensure that subjects learned to discriminate between the naloxone training dose and the placebo vehicle, the drug letter code associated with the drug administration *was not revealed* until the end of the experimental session. Subjects had to meet an accuracy criterion of $\geq 80\%$ correct responding on four consecutive sessions in order to enter the testing phase. If this criterion was not met within 10 sessions, subjects were dismissed from the study.

2.3.3. Testing (phase 3)

Dose–effect curves for isradipine (0, 2.5, 5, 10 mg/70 kg, by mouth (p.o.) dextromethorphan (0, 30, 60 mg/70 kg, p.o.), each alone and in combination with the training dose of naloxone (0.15 mg/70 kg, i.m.), were determined. After the session was completed, subjects were informed only that it was a test and that the drug code would not be revealed. During this phase, subjects were informed that if they received a drug not precisely like either of the training conditions, only novel-appropriate responses would be reinforced (see Bickel et al., 1993). However, in actuality, subjects' bonus earnings during all test sessions were equal to the average earned on the preceding four test-of-acquisition sessions; that is, earnings were not contingent upon discriminative performance.

Test-of-acquisition sessions (i.e., administration of the training dose of naloxone or placebo) were interspersed among the test sessions to ensure that the training conditions still appropriately controlled responding. If the training drug stimuli failed to control the appropriate response in one of these tests-of-acquisition, two more test-of-acquisition sessions were conducted. If the training drug stimuli did not control the appropriate response in two sessions, additional test-of-acquisition sessions were added until the criterion for acquisition of the discrimination (i.e., four consecutive correct) was met again. The ratio of test to test-of-acquisition sessions was approximately 1:1.

2.4. Experimental session

Sessions were conducted 3–5 days per week, depending on subject and staff availability, and typically began between 8:30–10:30 a.m. The beginning of experimental sessions remained consistent within subjects, who typically remained in the laboratory for approximately 3 h 36 m. A baseline field sobriety test was conducted at the beginning and end of each experimental session. Subjects were instructed to (1) count backwards from 100 by a specified number; (2) touch their nose with their index finger with eyes closed; (3) walk seven steps forwards and backwards "from heel to toe"; (4) stand on each foot for 30 s with eyes closed; (5) complete the digit symbol substitution test (DSST) on a computer; and (6) undergo an alcohol breathalyzer test. A predrug assessment cycle followed, consisting of baseline self-report questionnaires (see Section 2.5.2). Vital signs (blood pressure, heart rate) and body temperature were taken. Immediately afterward, one capsule was administered (-110 min), followed by a second capsule 30 min later (-80 min). Eighty minutes after the second capsule was administered, an injection was given into the muscle of the upper arm (0 min). Subjects completed tasks during two post-drug assessment cycles, conducted 20 and 40 min after the injection (Preston et al., 1987). Each assessment cycle lasted approximately 10 min and consisted of discrimination measures, self-reported measures on drug effect and vital signs (see Section 2.5). After the second post-injection assessment cycle was completed, a sealed envelope was opened for each subject, informing subject and experimenter either of the letter code of the administered drug or that the

session had been a test day. Subjects were then escorted to the adjacent room to recover from any drug effects. At this time, subjects were given food and were permitted to smoke outside of the building.

Subjects 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, subjects were instructed to maintain a regular pattern of smoking for the duration of the study. No food or beverage, except water, was allowed during the experimental session.

2.5. Dependent measures

2.5.1. Discrimination measures

Two discrimination performance procedures, the discrete choice task and the point distribution task, were collected in fixed order during each post-injection assessment (Preston et al., 1987, 1989; Bickel et al., 1989). In the discrete choice task, subjects made a discrete choice response that indicated by letter code (e.g., A, B, or "N") the drug they received. In the point distribution task, subjects distributed 50 points among the two drug codes and novel-response option depending upon how certain they were of the identity of the drug administered. In the point distribution task, only correct responses during training or test-of-acquisition sessions were converted to monetary reinforcement for subjects. Subjects earned up to US$9.00 per session or US$4.50 per point distribution procedure for maximal correct responding, such that each point on the correct code was worth US$0.09.

2.5.2. Self-report measures

Three questionnaires were administered on paper completed with a pen: the shortened version of the Addiction Research Center Inventory (ARCI), an adjective rating scale and visual analog scales (VAS). The ARCI consisted of 49 true/false questions that were scored as five subscales: 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 rating scale 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 subscales: (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 Agonist/Antagonist Scale, consisting of the terms confused, depressed, floating, headache, lightheaded, and numb (Preston et al., 1987).

The VAS 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 letter code) or dissimilar to either condition (identified by the letter "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.'

At the end of the second post-drug assessment cycle, subjects also completed a pharmacological drug class questionnaire, in which they indicated which type of drug they thought they had received from the following list: placebo (blank or nothing), opiates (heroin, methadone, etc.), phenothiazines (Haldol, major tranquilizers), barbiturates and sleeping medications, antidepressants (desipramine, imipramine), opioid receptor antagonists (naloxone, naltrexone), hallucinogens (marijuana, mushrooms, etc.), benzodiazepines (Xanax, Halcion, Valium, etc.), stimulants (cocaine, amphetamines, etc.), or phencyclidine (PCP, angel dust) (Preston et al., 1987). Subjects were not specifically educated about the different pharmacological drug classes listed on this questionnaire.

2.5.3. Physiological measures

Heart rate and blood pressure were taken at −130, −40, 0, 20, 40, 60, 110 min. Temperature was taken at −130, −40, 0, 10, 20, 30, 40, 50, 60. Heart rate and blood pressure were measured with a blood pressure cuff automated through a Dinamap Critikon 1846SX Vital Signs monitor (Shelton, CT); body temperature was measured by Thermoscan Instant Thermometer Pro-1 (San Diego, CA) and respiration rate was counted manually by the research nurse.

2.6. Drugs

Subjects were maintained on methadone hydrochloride at an average dose of 72.4 mg/day (range: 45–100 mg) by the opioid maintenance clinic they were attending. Subjects continued attending their respective opiate maintenance treatment facility during and after their participation in the study. Before entering the study, each subject was maintained at the same dose for at least 1 month. Subjects received their opioid maintenance dose at their respective clinics prior to attending each experimental session. The time between opioid maintenance dose and naloxone administration typically ranged from 1 h 54 m to 4 h 18 m across subjects.

The training dose of naloxone and naloxone placebo were administered via i.m. injection in a mean volume of 0.44 ml (range: 0.27–0.80). Injection volumes remained consistent

within individuals unless a subject's weight changed by more than 5 lb, in which case, injection volumes were adjusted. Isradipine and dextromethorphan were administered orally via blue opaque capsules. Drugs were prepared by the VA CT Healthcare System Research Pharmacy (West Haven Campus) for naloxone hydrochloride (Amerisource, Springfield, MA), isradipine (Amerisource), and dextromethorphan hydrobromide (Spectrum, New Brunswick, NJ). Active naloxone was prepared in 0.9% NaCl. Naloxone placebo consisted of 5% dextrose and 0.9% NaCl in a ratio of 1:1. Isradipine and dextromethorphan placebo consisted of lactose. Naloxone was injected 20 min prior to the first post-drug assessment cycle (Preston et al., 1987). Isradipine was administered 2.2 h prior to the first post-injection assessment cycle, because the peak plasma levels of isradipine occur at approximately 1.5 h post-administration (Clifton et al., 1988; Fitton and Benfield, 1990). Dextromethorphan was administered 1.7 h prior to the first post-injection assessment cycle, because its peak plasma levels occur at approximately 2.0 h post-administration (Silvasti et al., 1987; Kazis et al., 1996). Each drug was administered in a double-blind and "double-dummy" fashion.

The order of dose–effect curve determinations for those entering the testing phase was either dextromethorphan, dextromethorphan plus naloxone, isradipine, isradipine plus naloxone or isradipine, isradipine plus naloxone, dextromethorphan, dextromethorphan plus naloxone. The drug–naloxone combination always followed the drug alone condition for safety reasons. Order of dose–effect curve determinations and dose presentation within dose–effect curve determinations varied non-systematically across subjects. Although initially dextromethorphan was going to be tested at 30, 60 and 120 mg/70 kg, the 120 mg/70 kg dose of dextromethorphan was eliminated from the study after the subject who received this dose first withdrew from the study due to disliking the drug effects.

2.7. Data analyses

Data for only those subjects who completed at least one set of dose–effect curve determinations (i.e., test drug alone and test drug combined with naloxone) were used in the analyses. Discrimination data within each session were averaged across the two post-drug assessment cycles and reported as percentage of drug-appropriate responding. Results from the ARCI, VAS and adjective rating scales as well as physiological measures are reported as the mean change from predrug scores.

Repeated measures analyses of variance (ANOVA) were used to evaluate the significance associated with differences between the naloxone training dose and placebo on self-report and physiological measures during training/test-of-acquisition phases. Factors for self-report and physiological measures included training condition (naloxone and placebo) and session (four sessions each with placebo and naloxone).

During the testing phase, the significance of dose effects on discrimination, self-reports and physiological measures was evaluated for isradipine alone (0, 2.5, 5, 10 mg/70 kg), dextromethorphan alone (0, 30, 60 mg/70 kg), the naloxone dose in combination with isradipine (naloxone at 0.15 mg/70 kg plus 2.5, 5, 10 mg/70 kg of isradipine), and the naloxone training dose alone and in combination with dextromethorphan (naloxone at 0.15 mg/70 kg plus 30, 60 mg/70 kg of dextromethorphan). Placebo data used in these analyses were the averaged data across all placebo sessions during testing. Naloxone training dose data used in the analyses described above were the averaged data across all naloxone training sessions during testing. Self-report and physiological data for each dose–effect curve determination were entered into a repeated measures ANOVA with dose and post-drug assessment cycle as factors. Discrimination data were entered into a repeated measures ANOVA with dose as the factor. Of interest was to determine whether dose-related changes in a dependent measure occurred in a consistent direction. Thus, because dose represents a quantitative factor, orthogonal polynomials were used to partition the dose effect into linear, quadratic, cubic, etc., components (Winer, 1962). A significant linear or quadratic effect was interpreted as evidence of a dose-related increase (or decrease) in the outcome measure.

Since identical isradipine and dextromethorphan doses were tested in the absence and presence of the naloxone training dose, self-report and physiological data were each entered into a 2×3 or 2×2 repeated measures ANOVA with dose–effect curve determination (test drug alone vs. test drug plus naloxone) and test drug dose (2.5, 5, 10 mg/70 kg for isradipine and 30, 60 mg/70 kg for dextromethorphan) as factors. For all statistical analyses, $P < 0.05$ was used to infer statistical significance. Analyses were performed using SPSS statistical software.

3. Results

3.1. Discrimination performance during training/test-of-acquisition and testing

Because performance under both the discrete choice and point distribution discrimination tasks was similar, only data collected under the point distribution procedure will be presented. Five of seventeen subjects did not complete enough sessions to determine whether they met the criterion for discrimination (i.e., $\geq 80\%$ correct drug code identification across four consecutive sessions). Reasons for discontinuing participation included finding a job ($N = 1$), noncompliance with study protocols ($N = 3$), and elevated liver function tests ($N = 1$). Of the 12 subjects continuing through the test-of-acquisition phase, all met the discrimination criterion within a mean of 4.9 sessions (range: 4–8). Three of these twelve subjects were female, seven were Caucasian, four were African American, and one was

Hispanic. Eleven subjects were regular tobacco smokers. These subjects were maintained on methadone at 74.6 mg/day (range: 50–100 mg).

Of the 12 subjects who entered the third phase, 7 completed at least one set of dose–effect curve determinations (i.e., test drug alone and combined with naloxone) and 6 completed the entire phase, such that seven completed the isradipine dose–effect curve set and six subjects completed the dextromethorphan dose–effect curve set. Reasons for early termination of participation during this phase included disliking the drug effects ($N=2$), disruptive behavior ($N=1$), sustaining an injury unrelated to study participation ($N=1$), and being noncompliant with study protocols ($N=2$).

3.2. Effects of naloxone on self-report and physiological measures during training and test-of-acquisition

A main effect of drug occurred on the PCAG ($F(1,6)=6.2$, $P=0.05$) and LSD ($F(1,6)=8.6$, $P=0.03$) subscales of the ARCI, such that naloxone increased ratings relative to placebo (2.4 ± 0.9 vs. 0.4 ± 0.3 and 3.0 ± 0.8 vs. 0.1 ± 0.3, respectively). Naloxone produced significant increases in VAS ratings of 'any drug effect' (31.0 ± 6.9 vs. 8.8 ± 3.4, $F(1,6)=8.8$, $P=0.02$), 'bad effects' (31.9 ± 9.2 vs. 5.4 ± 4.0; $F(1,6)=12.6$, $P=0.02$), and 'like naloxone' (100 ± 0.0 vs. 0 ± 0) relative to placebo. Placebo significantly increased ratings of 'like placebo' relative to naloxone (0 ± 0 vs. 99.6 ± 0.4). Naloxone significantly increased ratings on the opioid receptor antagonist subscale of the adjective rating scale relative to placebo (1.6 ± 0.7 vs. -0.8 ± 0.4; $F(1,6)=7.6$, $P=0.03$). Naloxone significantly decreased ratings on the opioid agonist subscale of the adjective rating scale relative to placebo (-2.7 ± 1.0 vs. 0.4 ± 0.8; $F(1,6)=18.0$, $P=0.005$).

On the pharmacological drug class questionnaire, placebo was identified primarily as "placebo" on 18 of 28 occasions (64%), "barbiturate/benzodiazepine" on seven occasions (25%), "opiate" on two occasions (7.2%), and "antidepressant" on one occasion (3.6%). In contrast, the training dose of naloxone was identified as "opioid receptor antagonist" on 19 of 28 occasions (68%), "phenothiazine" on three occasions (10.7%), "placebo" on three occasions (10.7%), "barbiturate/benzodiazepine" on one occasion (3.6%), "stimulant" on one occasion (3.6%), and "opiate" on one occasion (3.6%). Physiological measures did not differ across the training conditions.

3.3. Effects of isradipine alone and dextromethorphan alone on discrimination, self-report and physiological measures

The effects of isradipine and dextromethorphan on discrimination performance are shown in Fig. 1. Isradipine alone produced minimal increases in naloxone-appropriate responding ($F(1,6)=1.3$, $P=0.3$) and a trend toward increases in 'novel'-appropriate responding ($F(1,6)=3.7$, $P=0.1$; Fig. 1, top left panel). Dextromethorphan alone produced minimal increases in naloxone-appropriate responding ($F(1,6)=1.0$, $P=0.4$) and increases in 'novel'-appropriate responding that were not significant ($F(1,6)=1.9$, $P=0.2$; Fig. 1, top right panel).

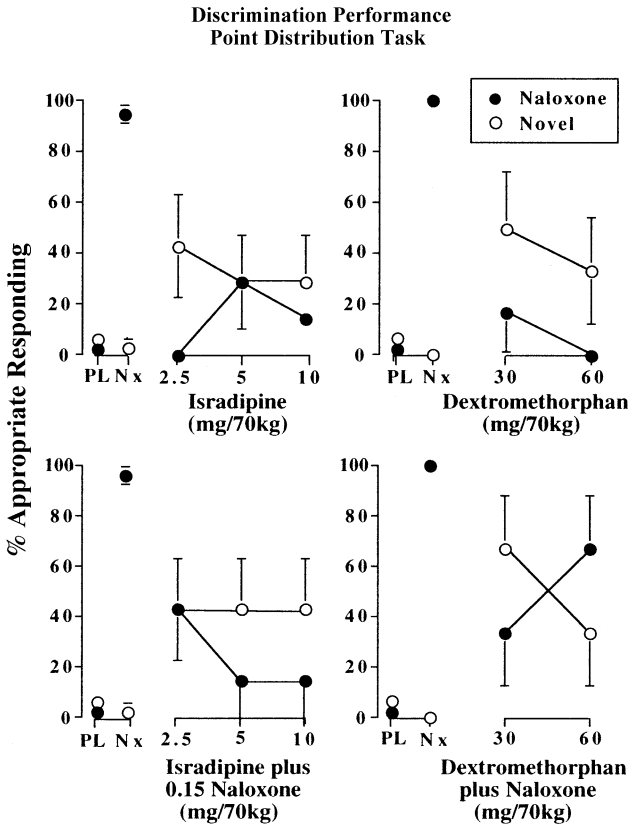

Fig. 1. The effects of isradipine alone (top left panel) or dextromethorphan alone (top right panel) and isradipine (bottom left panel) or dextromethorphan (bottom right panel) in combination with the naloxone training dose on discrimination performance under the point distribution task. Abscissa: Dose of drug in mg/70 mg bodyweight. Ordinate: Percentage naloxone-(filled circles) or 'novel'-(open circles) appropriate responding. Each point represents the mean across participants. Each bar represents standard error of the mean. Points above "P" and NX" represent means of participants across placebo and naloxone training days, respectively, during the test phase.

Those self-report and physiological measures that showed a trend toward or a main effect of dose for isradipine or dextromethorphan are shown in Table 1. Isradipine and dextromethorphan produced a trend toward increases and decreases, respectively, in VAS ratings of "high." Both isradipine and dextromethorphan significantly decreased VAS ratings of 'like placebo.' Isradipine, but not dextromethorphan, produced a trend toward increases in VAS ratings of 'like novel' and decreases in systolic blood pressure and a significant decrease in diastolic blood pressure.

Results of the pharmacological drug class questionnaire during testing are shown in Table 2. Isradipine was identified primarily as placebo at the lowest and highest dose and otherwise identified as coming from no specific drug class.

Table 1
Summary of self-report and physiological measures during testing of each agent alone[a]

Measure[b]	Isradipine						Dextromethorphan				
Dose[c]	0	2.5	5	10	*F*(*df*)	*p*	0	30	60	*F*(*df*)	*p*
VAS											
High	3.8 (1.8)	1.8 (1.7)	5.8 (4.5)	10.4 (5.2)	4.8 (1,6)	0.07	4.5 (2.0)	4.6 (2.9)	0.3 (0.3)	4.7 (1,5)	0.08
'Like Placebo'	90.0 (2.6)	57.1 (20.2)	42.9 (20.2)	57.1 (20.2)	6.5 (1,6)	*0.04*	88.4 (2.3)	33.3 (21.1)	66.6 (21.1)	6.2 (1,5)	*0.05*
'Like Novel'	5.9 (2.8)	42.8 (20.2)	28.6 (18.4)	28.6 (18.4)	3.7 (1,6)	0.1	6.8 (3.1)	50.0 (22.4)	33.3 (21.1)	1.9 (1,5)	0.22
Blood pressure											
Systolic	− .8 (6.5)	− 7.2 (4.2)	− 6.9 (5.6)	− 17 (3.0)	4.6 (1,6)	0.08	− 1.1 (7.7)	− 6.4 (6.0)	− 5.8 (8.0)	0.2 (1,5)	0.66
Diastolic	− 1.0 (4.5)	− 0.9 (3.1)	− 7.1 (3.2)	− 15.1 (3.1)	19.4 (1,6)	*0.005*	1.1	− 6.8 (4.6)	1.7 (4.7)	0.01 (1.5) (2.1)	0.91

[a] Only those measures in which at least one agent showed a trend toward or a linear or quadratic main effect of dose are shown.
[b] Each number represents the mean (± S.E.) of seven subjects for isradipine data and six subjects for dextromethorphan data.
[c] Dose of drug in mg/70 kg.

Dextromethorphan was identified primarily as placebo and otherwise as an opioid receptor antagonist at the lowest dose and as coming from no specific drug class at the high dose.

3.4. Effects of the naloxone training dose in combination with isradipine or dextromethorphan on discrimination, self-report and physiological measures

The effects of the naloxone training dose alone and in combination with isradipine or dextromethorphan are shown in Fig. 1. Relative to the naloxone training dose alone, which produced approximately 96% naloxone-appropriate responding, isradipine in combination with the training dose of naloxone produced a significant decrease in naloxone-appropriate responding, occasioning 43% and 14% naloxone-appropriate responding at the lowest dose and two highest doses, respectively ($F(1,6) = 27.3$, $P = 0.002$). Relative to the naloxone training dose, which produced minimal 'novel'-appropriate responding, naloxone in combination with isradipine produced a nonsignificant increase in 'novel'-appropriate responding to 43% at all doses of isradipine ($F(1,6) = 2.4$, $P = 0.17$; Fig. 1, bottom left panel).

Relative to the naloxone training dose alone, which produced approximately 96% naloxone-appropriate responding, dextromethorphan in combination with the training dose of naloxone produced a significant decrease in naloxone-appropriate responding, occasioning 33% and 66% naloxone-appropriate responding at the low and high doses, respectively ($F(1,5) = 7.5$, $P = 0.04$). Although the naloxone training dose produced minimal 'novel'-appropriate responding, naloxone in combination with dextromethorphan produced a significant increase in 'novel'-appropriate responding. Specifically, the low and high doses of dextromethorphan produced 66% and 33% 'novel'-appropriate responding, respectively ($F(1,5) = 7.5$, $P = 0.04$; Fig. 1, bottom right panel).

The effects of naloxone alone and in combination with isradipine or dextromethorphan on selected self-report and

Table 2
Subject ratings on the pharmacological drug class questionnaire during testing[a]

Dose[b]	Placebo	Opiate	Phenothiazine	Barbiturate	Antidepressant	Opiate	Hallucinogen	Benzodiazepine	Stimulant	Phencyclidine
	Antagonist									
Isradipine (n = 7)										
2.5	4	1	0	1	0	1	0	0	0	0
5.0	2	1	1	0	2	1	0	0	0	0
10.0	4	1	1	1	0	0	0	0	0	0
Dextromethorphan (n = 6)										
30	4	0	0	0	0	2	0	0	0	0
60	3	0	0	0	0	1	1	1	0	0
Isradipine + 0.15 mg/70 kg naloxone (n = 7)										
2.5	1	1	0	0	0	4	0	0	0	1
5.0	2	0	0	1	0	1	0	0	1	2
10.0	3	0	0	2	0	2	0	0	0	0
Dextromethorphan + 0.15 mg/70 kg naloxone (n = 6)										
30	0	0	0	0	0	4	1	1	0	0
60	0	0	0	0	0	5	1	0	0	0

[a] Each value represents the number of subjects identifying each particular drug/dose as being from a particular drug class.
[b] mg/70 kg.

physiological measures are shown in Figs. 2 and 3. Isradipine, but not dextromethorphan, significantly reduced naloxone-induced increases in ratings on the opioid receptor antagonist subscale of the adjective rating scale ($F(1,6)=5.7$, $P=0.05$ vs. $F(1,5)=0.01$, $P=0.9$; Fig. 2, top panels) and on the PCAG subscale of the ARCI ($F(1,6)=9.6$, $P=0.02$ vs. $F(1,5)=0.9$, $P=0.4$; Fig. 2, top middle panels). Relative to naloxone alone, isradipine combined with the naloxone training dose significantly decreased systolic ($F(1,6)=9.7$, $P=0.02$; Fig. 2, bottom middle panels) and diastolic

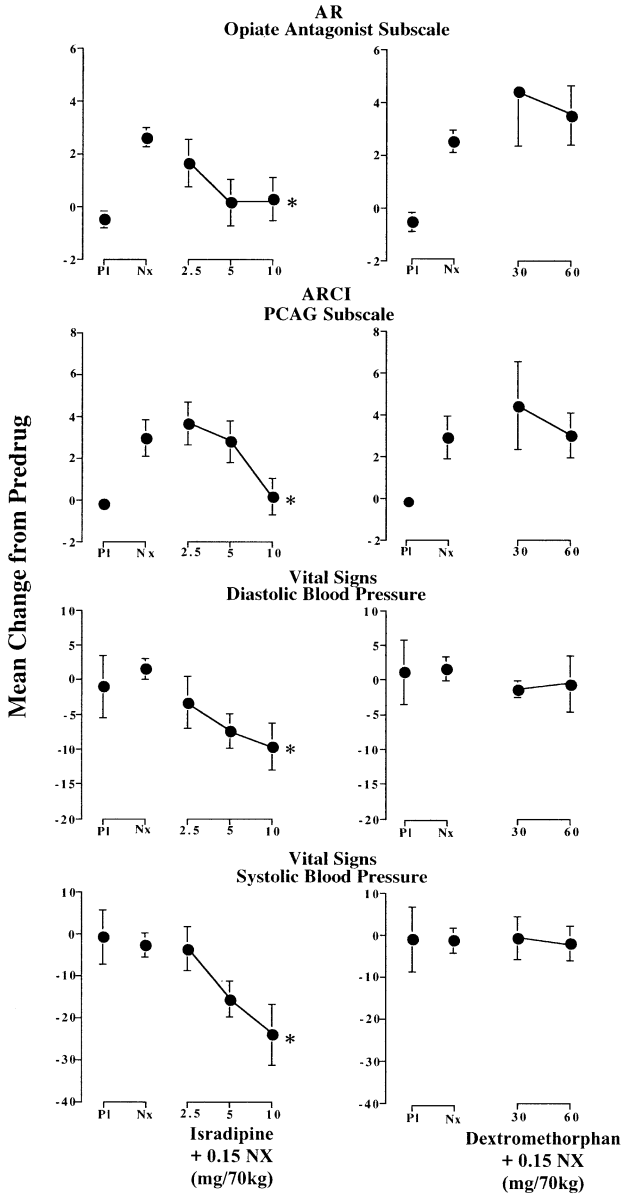

Fig. 2. The effects of naloxone alone and in combination with increasing doses of isradipine (left panels) or dextromethorphan (right panels) on selected self-report measures. Abscissa: Dose of drug in mg/70 mg bodyweight. Ordinate: Change from predrug. Each point represents the mean across participants. Each bar represents standard error of the mean. Points above "NX" represent means of participants across naloxone training days during the test phase. "AR" is Adjective Rating Scale.

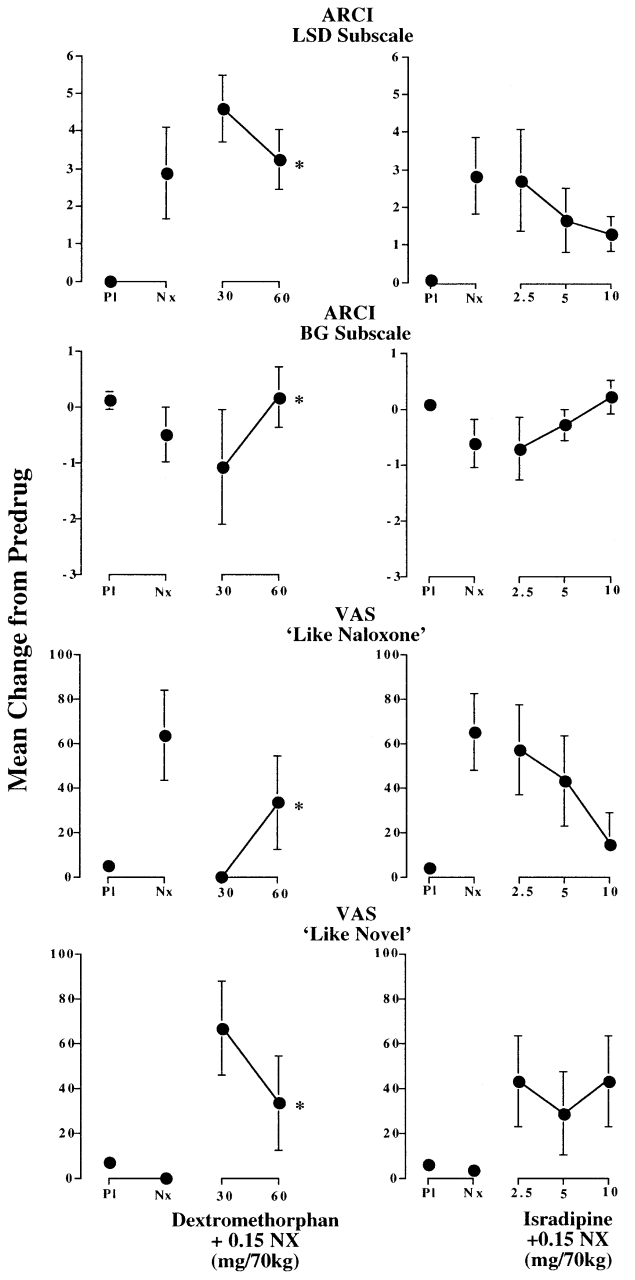

Fig. 3. The effects of naloxone alone and in combination with increasing doses of dextromethorphan (left panels) or isradipine (right panels) on selected self-report measures. Abscissa: Dose of drug in mg/70 mg bodyweight. Ordinate: Change from predrug. Each point represents the mean across participants. Each bar represents standard error of the mean. Points above "NX" represent means of participants across naloxone training days during the test phase.

($F(1,6)=13.9$, $P=0.01$; Fig. 2, bottom panels) blood pressure while dextromethorphan combined with the naloxone training dose did not ($F(1,5)=0.05$, $P=0.8$ and $F(1,5)=0.3$, $P=0.6$, respectively).

Relative to naloxone alone, naloxone in combination with dextromethorphan, but not isradipine enhanced ratings on the LSD subscale ($F(1,5)=15.9$, $P=0.01$ vs. $F(1,6)=2.7$, $P=0.15$) and decreased ratings on the BG

subscale ($F(1,5)=7.0$, $P=0.046$ vs. $F(1,6)=2.7$, $P=0.15$) of the ARCI (Fig. 3, top and top middle panels). Dextromethorphan in combination with the naloxone training dose significantly decreased VAS ratings of 'like naloxone' ($F(1,5)=7.7$, $P=0.04$; Fig. 3, bottom middle left panel) and increased VAS ratings of 'like novel' ($F(1,5)=7.5$, $P=0.04$; Fig. 3, bottom left panel) relative to naloxone alone. Isradipine combined with naloxone did not significantly alter these ratings relative to naloxone alone ($F(1,6)=2.4$, $P=0.17$; Fig. 3, bottom middle right panel and $F(1,6)=2.4$, $P=0.018$; Fig. 3, bottom right panel).

Results of the pharmacological drug class questionnaire during testing are shown in Table 2. When naloxone was administered in combination with isradipine, the number of subjects identifying naloxone as an opioid receptor antagonist was inversely related to isradipine dose, while the number of subjects identifying naloxone as placebo was positively related with isradipine dose. When naloxone was administered concomitantly with dextromethorphan, it was identified primarily as opioid receptor antagonist at both doses of dextromethorphan.

3.5. Isradipine or dextromethorphan alone vs. Isradipine or dextromethorphan in combination with the naloxone training dose on self-report and physiological measures

There was a main effect of isradipine dose–effect curve on two measures, such that isradipine plus naloxone produced lower scores on the opioid agonist subscale of the adjective rating scale (-2.4 ± 0.6 vs. 0.2 ± 1.0; $F(1,6)=6.5$, $P=0.04$) and on VAS ratings of 'like placebo' (27.8 ± 10.2 vs. 55.6 ± 18.6; $F(1,6)=7.4$, $P=0.04$) than isradipine alone. There was also a main effect of dextromethorphan dose–effect curve on two measures, such that dextromethorphan plus naloxone produced higher scores on the opioid receptor antagonist subscale of the adjective rating scale (4.0 ± 1.4 vs. 1.5 ± 1.1; $F(1,5)=11.0$, $P=0.02$) and on the LSD subscale of the ARCI (3.9 ± 0.8 vs. 1.3 ± 0.8; $F(1,5)=6.2$, $P=0.055$) relative to dextromethorphan alone.

There were no dose–effect curve × dose interactions for isradipine and one for dextromethorphan, such that dextromethorphan plus naloxone decreased scores on the A subscale of the ARCI to a greater extent at the 30 than 60 mg/70 kg dose relative to dextromethorphan alone ($F(1,5)=23.4$, $P=0.005$; data not shown).

4. Discussion

The results of this study were the following: (1) isradipine and dextromethorphan produced minimal naloxone appropriate responding and few, if any, significant self-reported or physiological effects; (2) isradipine significantly attenuated naloxone-occasioned responding without significantly increasing novel-appropriate responding and attenuated some of naloxone's behavioral effects; and (3) dextromethorphan significantly reduced naloxone-occasioned responding while significantly increasing novel-appropriate responding and enhanced naloxone-induced changes on a few self-reports.

The result that the naloxone stimulus generalized to neither isradipine nor dextromethorphan suggests that the naloxone discrimination has pharmacological specificity, which is consistent with previous naloxone discrimination studies in opioid-treated humans (Oliveto et al., 1998, 2002, 2003a,b; Preston et al., 1987). Although the lack of naloxone-appropriate responding by these agents occurred along with minimal changes in self-reported effects, isradipine did produce a trend toward increases in novel-appropriate responding. In addition, both isradipine and dextromethorphan significantly decreased ratings of 'like placebo,' indicating that these drugs did produce pharmacologically discernable effects. Reasons for a paucity of significant self-reported effects by these drugs may include lacking statistical power given the small sample size, employing self-reports that may not be sensitive to the behavioral effects of these compounds, or examining doses that were too low, particularly for dextromethorphan. For instance, participants receiving acute doses or a short course of isradipine have reported headache, dizziness, tachycardia, nausea and tinnitus (e.g., Christensen et al., 1993; Clifton et al., 1988). In methadone-stabilized subjects, dextromethorphan at doses much higher than examined here produced significant changes on several Profile of Mood States subscales (Rosen et al., 1996), which were not used in the present study. Because of the length of this study and the within-subject design used, when the first subject to receive the 120 mg/70 kg dose of dextromethorphan decided not to continue their participation because of disliking the drug effect, we decided to eliminate this dose from further study in order to increase the likelihood of study completion.

Nevertheless, isradipine did attenuate naloxone-occasioned responding without increasing novel-appropriate responding and attenuate several naloxone-induced changes in self-reported effects, suggesting that isradipine blocks the behavioral effects of naloxone in opioid-dependent humans. This finding is consistent with the nonhuman literature, in that calcium channel blockers have been shown to inhibit naloxone withdrawal contractures in the guinea-pig ileum either after morphine exposure (Valeri et al., 1990) or from morphine-dependent nonhumans (Alfaro et al., 1990), and attenuate naloxone- or naltrexone-precipitated signs of opiate withdrawal in opioid-dependent rodents (Bongianni et al., 1986; Colado et al., 1993; Kishioka et al., 1996; Krystal et al., 1996; Silverstone et al., 1992; Tokuyama et al., 1996).

A few studies in opioid-dependent humans have examined the effects of calcium channel blockers (Jimenez-Lerma et al., 2002; Rosen et al., 1994). For instance, opioid-dependent patients undergoing opiate detoxification with the calcium channel blocker nimodipine in combination with an opioid agonist manifested significantly fewer

opiate withdrawal symptoms than those treated with the opiate agonist plus a benzodiazepine or with α_2-adrenoceptor agents and naltrexone (Jimenez-Lerma et al., 2002). However, in a naloxone precipitated-withdrawal study in opioid-dependent humans, nimodipine at doses up to 90 mg, p.o., did not significantly decrease withdrawal ratings (Rosen et al., 1994). The reason for nimodipine's lack of efficacy is unclear, but may be due to testing doses too low to be efficacious, as the authors suggest.

In the present study, isradipine blocked the behavioral effects of naloxone in a manner similar to clonidine (Oliveto et al., 2003a), in that both attenuated naloxone-occasioned responding without increasing novel-appropriate responding, both attenuated naloxone-induced changes on several self-report measures, including opioid receptor antagonist-like effects, and both decreased the number of subjects identifying naloxone as an opioid receptor antagonist. The major difference between these two agents is that the effects of isradipine, but not clonidine, appeared to be dose-related. The reason for this difference may be that calcium channel blockers and α_2-adrenoceptor agonists influence the effects of an opioid receptor antagonist during opioid dependence via different mechanisms (e.g., Bongianni et al., 1986; Colado et al., 1993; Silverstone et al., 1992). For instance, while both calcium channel blockers and clonidine reduced naloxone-precipitated opiate withdrawal signs in opioid-dependent rats, clonidine, but not calcium channel blockers, altered naloxone-induced changes in serotonin metabolism (Colado et al., 1993) and naloxone-induced increases in norepinephrine (Silverstone et al., 1992). While the mechanism through which calcium channel blockers affect the behavioral effects of naloxone during opioid dependence is unclear, overall these results suggest that these agents would be useful in treating opiate withdrawal in humans.

In contrast to the results with isradipine, dextromethorphan attenuated the discriminative stimulus effects of naloxone while concomitantly increasing novel-appropriate responding, suggesting that dextromethorphan did not block the effects of naloxone as much as produce a discriminative stimulus effect not precisely like that of naloxone. Although NMDA receptor antagonists have been reported to disrupt discriminative control in nonhumans (e.g., Vosler et al., 2001), the effects of dextromethorphan in the present study are not consistent with loss of discriminative control. In the present study, dextromethorphan combined with naloxone produced either naloxone or novel-appropriate responding and no placebo-appropriate responding. If there had been a nonspecific impairment of discrimination performance, a more even distribution of responding would be expected to occur across all three response options.

Dextromethorphan also did not block, but actually enhanced naloxone-induced changes on several self-reports. These findings are consistent with the results of nonhuman drug discrimination studies (France and Woods, 1989; Holtzman, 1985). For instance, the NMDA receptor antagonist ketamine does not attenuate morphine abstinence-induced naltrexone-appropriate responding in morphine-treated rhesus monkeys trained to discriminate naltrexone from saline (France and Woods, 1989). In addition, dextromethorphan slightly attenuates naltrexone-occasioned responding in morphine-treated rats trained to discriminate naltrexone from saline (Holtzman, 1985). However, NMDA receptor antagonists have been shown to attenuate the development of morphine analgesic tolerance (Trujillo and Akil, 1994) as well as naloxone-precipitated opiate withdrawal in opioid-dependent nonhumans (e.g., Farzin, 1999; Higgins et al., 1992; Lizasoain et al., 1996; Popik and Skolnick, 1996; Tokuyama et al., 1996, 2001; Wang et al., 1995), which is inconsistent with the findings of the present study.

The effects of NMDA receptor agents on opiate withdrawal in opioid-dependent humans are also mixed (Bisaga et al., 2001; Koyuncuoglu and Saydam, 1990; Rosen et al., 1996, 1998). For instance, in opioid-dependent humans, neither the NMDA receptor antagonist lamotrigine (Rosen et al., 1998) nor dextromethorphan (Rosen et al., 1996) attenuated naloxone-precipitated withdrawal signs or symptoms. However, the NMDA receptor antagonist memantine did attenuate naloxone-precipitated withdrawal in opioid-dependent humans (Bisaga et al., 2001). In addition, opioid-dependent patients undergoing detoxification who received dextromethorphan plus diazepam reported significantly less opiate withdrawal than those who received chlorpromazine plus diazepam; however, because a control condition was not employed, the efficacy of dextromethorphan plus diazepam relative to placebo cannot be determined (Koyuncuoglu and Saydam, 1990). Overall these findings suggest that dextromethorphan may not be useful in the treatment of opioid withdrawal. However, given that these data are based on antagonist-precipitated withdrawal paradigms, which may differ from situations in which withdrawal occurs due to termination of opioid agonist administration, the efficacy of NMDA receptor antagonists in alleviating symptoms of a "naturalistic" opioid withdrawal as well as the role that NMDA receptor antagonists play in the expression of opioid withdrawal need to be clarified further.

In summary, the calcium channel blocker isradipine, but not the NMDA receptor antagonist dextromethorphan, blocked the discriminative stimulus and self-reported effects of naloxone in methadone-maintained humans trained to discriminate naloxone from placebo under a novel-response discrimination procedure. The results with isradipine were generally consistent with studies in opioid-dependent nonhumans and humans. The results with dextromethorphan were not consistent with the nonhuman literature, although results of studies in opioid-dependent humans have been mixed. These findings suggest that calcium channel blockers, but not NMDA receptor antagonists such as dextromethorphan, may be useful in the treatment of opiate withdrawal. More research is need to determine the possible efficacy of NMDA receptor antagonists in the treatment of opioid withdrawal.

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