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ORIGINAL INVESTIGATION

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Opiate withdrawal signs precipitated by naloxone following a single exposure to morphine: potentiation with a second morphine exposure

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Abstract Recent studies in humans with no prior history of opiate abuse indicated that naloxone-precipitated signs of opiate withdrawal could be observed after a single exposure to morphine, and that the severity of withdrawal was enhanced following a second morphine exposure 24 h later. The current study was conducted to establish a paradigm in rodents that resembled these conditions described in humans. To that end, naloxone-precipitated (0.03–3.0 mg/kg) suppression of operant response rates and somatic signs of withdrawal following single or repeated treatments with morphine (5.0 mg/kg) were assessed in previously opiate-naive rats. In one group of rats, naloxone was administered 4 h after both the first and second morphine pretreatment, while in a separate group of rats naloxone was administered 4 h after the second morphine pretreatment only. A single morphine pretreatment significantly increased naloxone's potency to suppress operant response rates, and resulted in the precipitation by naloxone of certain somatic signs of withdrawal. The effects of naloxone on both dependent measures (operant response rates and somatic signs) were potentiated following a second morphine pretreatment, regardless of whether naloxone was administered following both morphine exposures or only following the second morphine exposure. Thus, repeated morphine administration appears to be the critical factor underlying the progressive increase in antagonist potency, whereas prior experience with naloxone is not a necessary factor. The results provide additional support for the hypothesis that the development of dependence on opiates is a progressive phenomenon that may begin with a single dosing.

Key words Opiates · Morphine · Dependence · Acute dependence · Withdrawal · Abstinence · Naloxone

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Introduction

Dependence on opiates and the occurrence of withdrawal symptoms upon abstinence are most commonly studied under conditions of chronic exposure to opiate agonists. However, signs characteristic of the opiate withdrawal syndrome can be precipitated by injection of an opiate antagonist after pretreatment with a single dose of an opiate agonist. For example, studies in humans have indicated that administration of the opiate antagonist naloxone following exposure to a single dose of morphine (Jones 1980; Bickel et al. 1988; Heishman et al. 1989a,b, 1990; Azorlosa et al. 1994) or methadone (Wright et al. 1991) can produce physiological signs, subjective ratings and objective (somatic) symptoms characteristic of withdrawal from chronic opiates. This phenomenon has been referred to as "acute dependence" (Martin and Eades 1964; Bickel et al. 1988). The severity of naloxone-precipitated withdrawal in these studies has been shown to be dependent on the dose of opiate agonist (Jones 1980; Bickel et al. 1988), the dose of naloxone (Heishman et al. 1989a), and the interval between agonist pretreatment and naloxone administration (Heishman et al. 1989b, 1990; Kirby et al. 1990).

The observation of an acute dependence state as defined by antagonist-precipitation of somatic withdrawal signs following acute pretreatment with opiates has been reported in a number of other species, including monkeys (Krystal and Redmond 1983), dogs (Martin and Eades 1964; Jacob and Michaud 1974), hamsters (Schnur et al. 1992), mice (Cheney and

Goldstein 1971; Jacob et al. 1974; Kosersky et al. 1974; Smits 1975; Wiley and Downs 1979; Ritzmann 1981; Ramabadran 1983; Sofuoglu et al. 1990), and rats and gerbils (Ramabadran 1983). Many of these studies reported naloxone-induced jumping behavior in morphine-pretreated mice, and typically high doses of morphine (50–150 mg/kg) were used (e.g. Kosersky et al. 1974; Smits 1975; Wiley and Downs 1979; Ritzmann 1981; Ramabadran 1983). In some cases where lower doses of morphine (4–12.5 mg/kg) were employed (e.g. Smits 1975; Wiley and Downs 1979), very high doses of naloxone typically were administered (100–128 mg/kg). Jacob and Michaud (1974), however, reported that naloxone (3 mg/kg) could elicit clear signs of opiate withdrawal in dogs pretreated intravenously with 0.1 mg/kg morphine 1.5 h earlier, suggesting that this phenomenon can be observed following relatively small doses of opiate agonists if a high dose of antagonist is employed.

Suppression of operant responding in rats by low doses of opiate antagonists, well-characterized as a sensitive index of withdrawal from chronic opiates (Gellert and Sparber 1977; Adams and Holtzman 1990; Schulteis et al. 1994), also can be observed following treatment with a single dose of various opiate agonists (Meyer and Sparber 1977; Young 1986; Adam and Holtzman 1990; White-Gbadebo and Holtzman 1994). These studies have successfully established operant responding as a sensitive index of acute antagonist-precipitated withdrawal from morphine at agonist and antagonist doses considerably lower than those typically employed in the studies of somatic withdrawal signs (see above). One purpose of the current study was to determine whether somatic signs of withdrawal could be seen in the same morphine and naloxone dose ranges that produce reliable suppression of operant responding.

Interestingly, Adams and Holtzman (1990) reported that sensitivity to naltrexone-induced suppression of operant response rates increased with repeated weekly morphine treatments over a course of 2 months. In this and similar (White-Gbadebo and Holtzman 1994) studies, a complete naltrexone-dose effect function was determined following each morphine treatment using a cumulative dosing procedure, and it was suggested that repeated experience with naltrexone in the presence of morphine may have contributed to sensitization of antagonist effects through a process of conditioning. Recently it has been demonstrated in humans that withdrawal signs were more pronounced when naloxone was given for the first time following two morphine pretreatments spaced 24 h apart than when naloxone was given following the first morphine pretreatment (Azorlosa et al. 1994). These latter data would suggest that repeated experience with an opiate antagonist is not an obligatory factor in the development of enhanced sensitivity to that antagonist upon repeated morphine treatment. Thus, in an effort to develop an animal model which closely paralleled the phenomenon characterized by Azorlosa et al. (1994) in human subjects, a second purpose of these studies was to determine whether enhanced sensitivity to naloxone in the operant suppression paradigm could be observed with as few as two morphine treatments, and whether repeated naloxone experience was a necessary factor under such conditions.

To these ends, the effects of naloxone following one or two pretreatments with morphine were assessed in previously opiate-naive rats. Naloxone was administered either after both the first and second morphine pretreatment, or after the second morphine pretreatment only. Suppression by naloxone of food-reinforced operant responding served as the dependent measure. In addition, parallel studies were conducted using a rating scale for somatic withdrawal signs (Gellert and Holtzman 1978) as the dependent measure.

Materials and methods

Animals

Male Wistar rats (n = 143, Charles River, Kingston, N.Y., USA) weighing 350–450 g at the time of testing were used. All rats were group housed (2–3/cage) in a temperature- and humidity-controlled room with a 12-h light/12-h dark cycle. Rats trained in the food-reinforced operant paradigm were maintained on 15 g rat chow per day in addition to the food pellets earned in the operant boxes (total food intake was approximately 22 g/rat per day). Rats tested for somatic signs of opiate withdrawal had ad lib access to food, and all rats had ad lib access to water. All training and testing took place during the active (lights out) portion of the rats' daily activity cycle.

Drugs

Morphine sulfate was provided by the National Institute on Drug Abuse (Rockville, Md., USA), and naloxone HCl was purchased from Sigma (St Louis, Mo., USA). Both drugs were prepared for injection in physiological saline, and all injections were made subcutaneously (SC) in a volume of 0.1 ml/100 g body weight. Morphine was administered at a dose of 5 mg/kg, and naloxone was administered at one of several doses (0.03, 0.10, 0.30, 1.0 and 3.0 mg/kg). All doses are expressed as the salt. The 5 mg/kg dose of morphine was chosen based upon earlier reports (Adams and Holtzman 1990) and preliminary experiments (Schulteis, Heyser, and Koob, unpublished observations) which indicated that this dose of morphine produced little or no effect on response rates by itself at the time of antagonist administration and testing (4 h post-morphine). Vehicle injections consisted of physiological saline in a volume of 0.1 ml/100 g body weight. Each subject received only a single drug condition (either vehicle or one dose of naloxone).

Operant training

Seven operant chambers (Coulbourn Instruments, Columbus, Ohio, USA) served as the training and testing environments. Each chamber was equipped with a food hopper located 4 cm above the grid floor, a lever located to the right of the food hopper, and a cue light located above the lever. The cue light illuminated for 1 s as a

food pellet (45 mg) was delivered each time a rat completed a fixed-ratio (FR) component. As described previously (Koob et al. 1989; Schulteis et al. 1994), rats were autoshaped to lever press for food pellets in 30-min sessions 5 days a week, beginning on an FR-1 schedule and progressing to an FR-15 schedule (1-s timeout). Training continued until stable baseline response rates were achieved, with stability defined as less than 10% variation from the mean of 3 consecutive test days.

Following establishment of stable baseline operant response rates as described above (typically 3–5 weeks), daily testing consisted of two sessions separated by 4 h. The first session (10 min duration) was followed by an injection of vehicle or morphine, and the second session (20 min duration) was preceded 5 min by an injection of vehicle or naloxone. The initial 10-min session allowed for verification of stable response rates prior to any treatment; any significant (>15%) deviation in response rate for a given rat noted during this 10-min session resulted in a delay in treatment until appropriate baseline response rates could again be achieved for that rat. The 4-h interval between morphine pretreatment and naloxone administration was chosen based upon previous studies in rats (Young 1986; Adams and Holtzman 1990; White-Gbadebo and Holtzman 1994), and because it closely parallels the morphine-naloxone interval (4.33 h) employed by Azorlosa et al. (1994) in their study with humans.

Rats were tested in this manner for 5 consecutive days with a drug pretreatment/treatment regimen as detailed in Table 1. The first day (Monday) allowed rats to acclimate to the new testing schedule; the second day (Tuesday) rats received two vehicle injections, and this served as the baseline day. Response rates on the final 3 Experimental Days (Wednesday–Friday) were expressed as a percentage of this baseline response rate. Throughout these experiments, response rates on the Baseline Day averaged 110.8 responses/min (range 68.2–166.0 responses/min), and rats were counterbalanced to ensure equivalent baseline performance between groups prior to experimental manipulation.

Ratings of somatic signs of withdrawal

In a separate cohort of animals, morphine was administered 4 h prior to injection of vehicle or one of several doses of naloxone. Drug solutions were blind-coded so that the observer was unaware of the treatment given. As an overall index of withdrawal intensity, the weighted scale of Gellert and Holtzman (1978) was used. This scale consists of graded signs of weight loss, number of escape attempts, number of wet dog shakes, instances of abdominal constrictions, and checked signs (simply scored as present or absent) including diarrhea, facial fasciculations/teeth chattering, swallowing movements, profuse salivation, chromodacryorrhea, ptosis, abnormal posture, penile grooming/erection/ejaculation, and irritability upon handling. On the Gellert and Holtzman scale, graded signs with the exception of weight loss are assigned a weighting factor from 1 to 4 based on frequency of appearance, and checked signs receive values of 2–7 depending upon the particular withdrawal sign noted, but regardless of frequency of appearance. With the exception of weight loss, signs were observed for the first 10 min after injection. Weight loss was calculated as the difference between the weight determined immediately prior to naloxone administration and a second determination made 60 min after injection (no food was available to the rats during this interval). One point was assigned for each 1% of body weight lost.

Experiment 1: dose-effect functions for naloxone-precipitated suppression of operant responding for food

Experimental design and statistical analysis

Rats (n = 56) were trained and tested as described in the Materials and methods section, utilizing the drug pretreatment/treatment regimen outlined in Table 1. In this experiment, separate groups of rats were tested for each treatment condition (vehicle or naloxone 0.03, 0.10, 0.30, 1.0, 3.0 mg/kg; n = 8–10/group), with each rat receiving the same dose of naloxone (or vehicle) on 3 consecutive days (Saline Day, Morphine Day 1, Morphine Day 2). Data were analyzed using a two-factor mixed design ANOVA, with dose as a between-subjects factor and treatment day as a within-subjects factor. Subsequently, minimal effective doses of naloxone were determined through individual comparisons of the mean effect produced by each naloxone dose on a given treatment day to the mean observed in the vehicle control group on that day using the Dunnett's correction for multiple comparisons to a single control. In all cases the criterion for statistical significance was $P < 0.05$. Relative potency analysis (Tallarida and Murray 1986) provided an additional measure of shift in naloxone potency from Morphine Day 1 to Morphine Day 2.

Results

As illustrated in Fig. 1, the potency of naloxone to suppress operant responding for food was greater when animals were pretreated 4 h earlier with a single dose of morphine than when animals were pretreated with vehicle, and was greater still when naloxone was

Table 1 Summary of treatment regimen for experiments 1 and 2

	Experimental day	Pretreatment	Treatment (4 h later)	
			Experiment 1	Experiment 2
Monday	No treatments	–	–	–
Tuesday	Baseline Day	Vehicle	Vehicle	Vehicle
Wednesday	Saline Day	Vehicle	Naloxone*	Naloxone*
Thursday	Morphine Day 1	Morphine	Naloxone*	Vehicle
Friday	Morphine Day 2	Morphine	Naloxone*	Naloxone*

* Different groups of animals received different doses of naloxone (0, 0.03–3.0 mg/kg), but within a given group the dose of naloxone was held constant across Experimental days

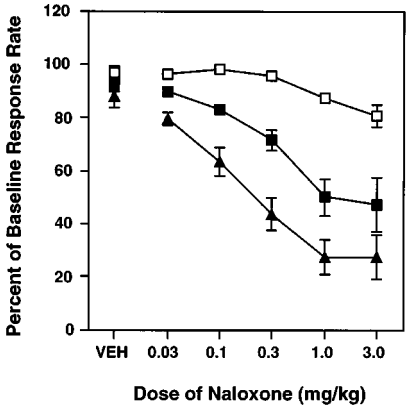

Fig. 1 Dose-response functions for naloxone-precipitated suppression of response rates on the Saline Day (*open squares*, □), Morphine Day 1 (*filled squares*, ■), and Morphine Day 2 (*filled triangles*, ▲). A significant shift to the left in the dose-response function was seen when naloxone was administered 4 h following morphine (5.0 mg/kg) on Morphine Day 1, with a further significant shift to the left in the dose-response function when naloxone followed morphine on Morphine Day 2 (see experiment 1 for further details). Values respresent percent of responding relative to Baseline Day (see Table 1). Different groups of rats (n = 8–10/group) were tested for each dose of naloxone, but the dose was held constant within a given group across Experimental Treatment Days

administered again following a second morphine pretreatment. Statistical analysis revealed a significant main effect of dose [$F(5,50) = 21.26$, $P < 0.0001$], a significant main effect of treatment day [$F(2,100) = 164.86$, $P < 0.0001$] and a significant dose × treatment day interaction [$F(10,100) = 9.29$, $P < 0.0001$].

Subsequent individual means comparisons (Dunnett's correction, $P < 0.05$) revealed that the minimal effective dose of naloxone to suppress response rates was 1.0 mg/kg on the Saline Day. A significant shift in minimal effective dose was observed on Morphine Day 1 (0.3 mg/kg). On Morphine Day 2 a further shift in minimal effective dose to 0.1 mg/kg naloxone was observed. The relative potency of naloxone on Morphine Day 2 as compared to Morphine Day 1 was calculated as 4.90, further confirming the increase in naloxone potency following the second morphine pretreatment.

Experiment 2: effect of naloxone history on naloxone-induced suppression of operant responding

The study by Azorlosa and colleagues (1994) revealed a greater increase in the potency of naloxone following two morphine pretreatments separated by 24 h than following a single morphine pretreatment. The results of experiment 1 suggest a similar phenomenon occurs in rats using suppression of operant responding for food as a dependent measure. However, the rats in experiment 1 received naloxone on both Morphine Day 1 and Morphine Day 2, whereas the human subjects in the experiment of Azorlosa et al. (1994) received naloxone only following the second morphine pretreatment. It is possible that repeated experience with naloxone under conditions of morphine pretreatment in experiment 1 contributed to the increased naloxone potency observed on Morphine Day 2. To examine this possibility, the effects of naloxone administered only on Morphine Day 2 were compared to the effects of naloxone in animals receiving the drug on both Morphine Day 1 and Morphine Day 2.

Experimental design

The drug pretreatment/treatment regimen again was as outlined in Table 1. The experimental design was similar to that described in experiment 1, with the following exceptions: 1) a narrower dose range of naloxone was examined (0.1, 0.3, 1.0mg/kg); and 2) rats (n = 30, 10/dose) received naloxone only on the Saline Day and again on Morphine Day 2. The effects of naloxone on Morphine Day 2 in these rats were compared to the effects of naloxone on Morphine Day 2 observed in experiment 1, wherein rats received naloxone on all 3 treatment days. Thus, the Morphine Day 2 data for rats treated with naloxone on both Morphine Day 1 and Morphine Day 2 were taken from the results of experiment 1 (for doses of 0.1, 0.3, and 1.0 mg/kg naloxone). Statistical analysis consisted of a two-factor between-subjects ANOVA with naloxone dose as one factor and morphine-naloxone history as the second factor.

Results

As shown in Fig. 2, naloxone history had no significant effect on the naloxone dose-effect function observed on Morphine Day 2. Rats receiving the morphine-naloxone combination for the first time on Morphine Day 2 showed comparable suppression in operant responding as rats that received naloxone on both days of morphine pretreatment. This was confirmed by two-factor ANOVA which indicated a significant main effect of naloxone dose [$F(2,54) = 11.15$, $P < 0.0001$], but no significant main effect of naloxone history [$F(1,54) < 1.0$, n.s.) or dose × history interaction [$F(2,54) = 1.40$, n.s.].

Experiment 3: naloxone-precipitated somatic withdrawal signs

This experiment was conducted to determine whether somatic signs of withdrawal could be seen in the same naloxone dose range that produced a reliable suppres-

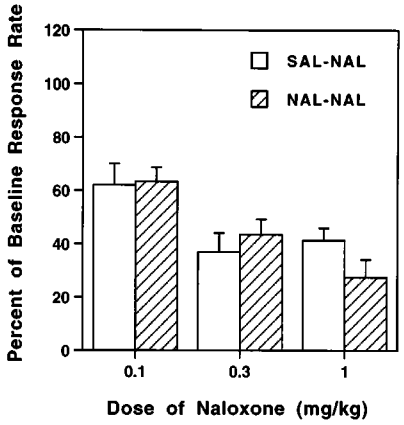

Fig. 2 The magnitude of naloxone-precipitated suppression of response rates on Morphine Day 2 was not dependent on morphine-naloxone history. Animals receiving naloxone for the first time on Morphine Day 2 (*SAL-NAL*, *open bars*) showed suppression of responding equal to that observed in animals receiving naloxone on both Morphine Day 1 and Morphine Day 2 (*NAL-NAL*, *striped bars*; data reproduced from experiment 1, Fig. 1). Values represent percent of responding relative to Baseline Day (see Table 1). Different groups of rats ($n = 10$/group) were tested for each dose of naloxone (see experiment 2 for further details)

sion of operant responding, and whether the somatic signs also would be more pronounced following a second morphine pretreatment.

Experimental design and data analysis

In this experiment, there were 2 treatment days, Morphine Day 1 and Morphine Day 2. On Morphine Day 1, rats ($n = 57$) were pretreated with morphine (5.0 mg/kg) and 4 h later were treated with vehicle or a dose of naloxone (0.03, 0.1, 0.3, 1.0, 3.0 mg/kg; $n = 8$–11/group) immediately prior to assessment of somatic withdrawal signs as detailed in the Materials and methods section. On Morphine Day 2, rats were again pretreated with morphine and 4 h later were tested after receiving the same treatment (vehicle or naloxone dose) administered on Morphine Day 1. The data for the overall withdrawal score computed according to the method of Gellert and Holtzman (1978; see also Schulteis et al. 1994) were subjected to mixed design ANOVA with dose of naloxone as a between-

Table 2 Summary of global rating and individual somatic signs of withdrawal as a function of morphine treatment day and dose of naloxone

		Vehicle ($n = 11$)	0.03 ($n = 8$)	0.10 ($n = 10$)	0.30 (mg/kg) ($n = 9$)	1.0 ($n = 11$)	3.0 ($n = 8$)	All doses ($n = 46$)
Global rating[a]	Day 1	1.44	2.80	4.26	5.28[d]	7.26	9.89	5.90[c]
	Day 2	1.96	4.54	5.50[d]	7.37	12.59	12.99	8.60[c]
Individual signs[b]								
Escape jumps (≥2)	Day 1	0	13	10	0	9	25	11
	Day 2	0	25	0	22	36	63	28
Diarrhea	Day 1	0	0	0	11	27	0	9
	Day 2	9	0	0	0	0	13	2
Teeth chattering	Day 1	27	25	60	78[d]	64	75	61[c]
	Day 2	46	63	70[d]	56	73	88	70[c]
Swallowing	Day 1	0	25	10	44[d]	64	88	46[c]
movements	Day 2	0	25	60[d]	78	82	88	67[c]
Profuse salivation	Day 1	0	0	0	0	0	13	2
	Day 2	0	0	0	11	36[d]	13	13[c]
Ptosis	Day 1	0	0	0	0	18	25	10
	Day 2	0	0	0	22[d]	9	50	15[c]
Penile	Day 1	0	50[d]	40	33	64	38	46[c]
grooming/ejaculation	Day 2	9	25	60[d]	11	64	63	46[c]
Irritability/	Day 1	18	0	10	44[d]	73	75	41[c]
vocalization	Day 2	0	50[d]	30	78	81	88	65[c]
Abnormal posture	Day 1	0	0	0	22[d]	36	38	20[c]
	Day 2	0	0	0	33[d]	9	38	15[c]

[a]Based upon weighted scale of Gellert and Holtzman (1978). Data reflect mean values
[b]Data reflect % of subjects showing sign in 10-min test
[c]Significant overall main effect of naloxone dose as determined by ANOVA (global rating) or information statistic (individual signs)
[d]Minimum effective dose (MED: $P < 0.05$ vs vehicle-treated controls). MED determined only for signs and treatment days where overall main effect of naloxone was significant
Note: signs which were not observed at any dose (weight loss >2.0%, abdominal constrictions, wet dog shakes, chromodacryorrhea) are not included in this table

subjects factor and treatment day as a within-subjects factor. In addition, the frequency of occurrence of individual somatic withdrawal signs was evaluated using the nonparametric information statistic test (Kullback 1968; Fray et al. 1980). Relative potency analysis (Tallarida and Murray 1986) again served as an additional index of shift in naloxone potency with repeated morphine pretreatment. An additional group of rats (n = 10) received the 0.3 mg/kg dose of naloxone only on Morphine Day 2. The effect of naloxone in these rats was compared to the effect of naloxone in rats treated with naloxone on both morphine days in a one-factor ANOVA.

Results

As shown in Table 2, naloxone produced a dose-dependent increase in the overall somatic withdrawal score on both Morphine Day 1 and Morphine Day 2, with a greater effect being observed following the second morphine pretreatment. This was confirmed by significant main effects of dose [$F(5,51) = 32.24$, $P < 0.0001$], a significant main effect of treatment day [$F(1,51) = 25.84$, $P < 0.0001$], and a significant dose × treatment day interaction [$F(1,51) = 2.61$, $P < 0.05$]. The minimal effective dose of naloxone for inducing a significant increase in the withdrawal score on Morphine Day 1 and Morphine Day 2 was 0.3 and 0.1 mg/kg, respectively. The relative potency of naloxone on Morphine Day 2 as compared to Morphine Day 1 was calculated to be 5.47, quite similar to the relative potency calculated for naloxone-induced suppression of operant responding (4.90) in experiment 1. Finally, rats receiving naloxone (0.3 mg/kg) only on Morphine Day 2 had a mean withdrawal score (mean = 7.94 ± 1.09) no different from animals receiving morphine on both days (mean = 7.37 ± 1.00), indicating that morphine-naloxone histroy did not affect withdrawal intensity [$F(1,17) < 1.0$, n.s].

Table 2 also presents a summary of the percentage of subjects showing individual somatic signs of withdrawal on Morphine Day 1 and Morphine Day 2 as a function of naloxone dose. Not all somatic signs of withdrawal could be elicited following either a single or a repeated pretreatment with 5.0 mg/kg morphine. For example, body weight loss > 2.0%, abdominal constrictions, wet dog shakes, and chromodacryorrhea were almost never observed in the current experiment (data not shown), even at doses of naloxone (0.3–3.0 mg/kg) which would elicit such signs in chronically dependent animals (for comparison see Blasig et al. 1973; Wei et al. 1973; Gellert and Holtzman 1978; Gold et al. 1994; Schultels et al. 1994). Signs such as escape jumps, diarrhea, profuse salivation, ptosis, and abnormal posture were infrequently observed on Morphine Day 1, although some increased in incidence on Morphine Day 2. Finally, signs such as teeth chattering, swallowing movements, penile erection/ejaculation, and irritability/vocalization were frequently observed on both Morphine Day 1 and Morphine Day 2, and most increased in incidence after the second morphine pretreatment.

Discussion

The current study provides further evidence that naloxone dose-dependently precipitates an opiate withdrawal-like reaction in rats treated acutely with a single dose of morphine (5 mg/kg). Both suppression of operant responding for food and a somatic withdrawal rating scale provided reliable indices of this opiate withdrawal-like phenomenon. These data support and extend previous reports of increased potency of opiate antagonists to suppress operant response rates following acute treatment with morphine at doses of 3–15 mg/kg (e.g. Meyer and Sparber 1977; Young 1986; Adams and Holtzman 1990; White-Gbadebo and Holtzman 1994). In addition, the results of the current study indicate an increased potency of naloxone following a second morphine exposure 24 h after initial morphine administration on both the operant response rate and somatic withdrawal sign dependent measures. These findings are consistent with the human data of Azorlosa and colleagues (1994), thereby validating an animal model that should prove useful in identifying the neural substrates mediating this acute dependence-like phenomenon.

Progressively increasing potency of opiate antagonists to suppress operant response rates in rodents following repeated exposure to opiates has been reported previously (Young 1986; Adams and Holtzman 1990), but under different conditions from those employed in the current study. For example, Adams and Holtzman (1990) have provided evidence that after repeated weekly morphine exposures for a period of 2 months to 1 year, the dose-response curve for naltrexone-induced suppression of response rates resembled that seen in chronically opiate-dependent animals. In this experiment, rats were treated once weekly with morphine, and the rate-suppressing effects of naltrexone were determined 4 h after each morphine pretreatment using a cumulative naltrexone dosing procedure in which the naltrexone dose was periodically increased until responding was completely suppressed. After 2 months of repeated weekly pretreatment with a 5.6 mg/kg dose of morphine, the dose-response curve for naltrexone was shifted 600-fold. In the present study, we report that a significant increase in naloxone potency can be observed with as few as two morphine pretreatments separated by 24 h.

Adams and Holtzman (1990) interpreted their findings of sensitization to the rate-suppressing effects of naltrexone with repeated morphine exposure as

consistent with the development of conditioning to the interoceptive stimuli produced by naltrexone. According to this view, the shift in the cumulative dose-response function for naltrexone seen after repeated exposures to morphine was the result of an association between the interoceptive stimuli produced by low doses of naltrexone (early in session) with the unconditioned rate-decreasing effects produced by larger doses of naltrexone (late in session). Consistent with this interpretation, it has been shown that animals trained to detect the discriminative stimulus properties of opiate antagonists generalize to much lower doses of antagonists if pretreated with morphine (France and Woods 1985, 1988).

However, in the present experiment, a potentiated effect of naloxone could be seen following as few as two morphine pretreatments. Moreover, the results of experiment 2 suggest that prior experience with naloxone in the presence of morphine was not necessary for the greater withdrawal severity precipitated following the second morphine exposure. In the study by Azorlosa and colleagues (1994), naloxone administered for the first time after the second morphine pretreatment also produced a greater incidence of withdrawal signs than naloxone administered after the first morphine pretreatment.

Finally, it was reported recently that rats pretreated with morphine on three separate occasions showed a progressive increase in sensitivity to naloxone even when successive morphine exposures were separated by intervals of 1, 3, or 6 weeks (Schulteis et al. 1995). Consistent with the results presented in the current manuscript, the progressive increase in naloxone sensitivity observed at 1- or 3-week morphine inter-treatment intervals was found to be independent of naloxone history. Somewhat surprisingly, even rats receiving naloxone after each of the three morphine pretreatments at 6-week inter-treatment intervals showed a progressive increase in naloxone sensitivity with each successive morphine exposure. However, with this 6-week inter-treatment interval, rats that received naloxone for the first time after the third and final morphine exposure showed no potentiated response to naloxone.

Thus, both animal and human data suggest that repeated experience with naloxone does not play an obligatory role in the development of increased sensitivity to naloxone with repeated morphine exposures separated by intervals of 24 h, 1 week, or 3 weeks. However, this does not imply that naloxone experience and conditioning factors are devoid of any possible role in the phenomenon of sensitization of antagonist effect following repeated agonist pretreatment. Indeed, the data summarized in the previous paragraph indicate that under certain conditions, such as extended (e.g. 6-week) intervals between successive morphine exposures, naloxone experience does appear to play an important role (Schulteis et al. 1995). This suggests an interesting parallel with studies of morphine tolerance which have shown that associative conditioning processes play a more prominent role in the development of tolerance when long as opposed to short intervals between successive morphine treatments are employed (Tiffany et al. 1992).

In addition, it remains quite possible that conditioning could have contributed to the dramatic (600-fold) increase in naltrexone sensitivity observed by Adams and Holtzman (1990) following 2 months of repeated weekly morphine-naltrexone exposures. With multiple associations in this paradigm, conditioning to the interoceptive stimulus properties of naltrexone or to the environment of the testing chamber would appear quite feasible, and it is possible that the degree of sensitization of naltrexone-induced suppression of operant response rates would not have been as great without the contribution of conditioning.

A further intriguing possibility is that conditioned stimuli could come to substitute for the effects of an opiate antagonist upon repeated administration of the antagonist following repeated morphine pretreatments. In that regard, it has been reported in opiate dependent humans (O'Brien et al. 1977), monkeys (Goldberg and Schuster 1967), and rats (Wikler and Pescor 1967; Baldwin and Koob 1993) that repeated pairings of discrete stimuli such as tones, lights, or smells with an opiate antagonist leads to a state of conditioned withdrawal, in which the stimuli themselves are capable of eliciting withdrawal-like signs similar to those precipitated by the antagonist. It would be interesting to ascertain whether a similar phenomenon can be observed when naloxone is paired with discrete stimuli in animals given repeated intermittent exposure to moderate doses of morphine in a model such as that employed in the current study and by others (Young 1986; Adams and Holtzman 1990).

It should be noted that increased sensitivity to the rate-decreasing effects of opiate antagonists can be seen following repeated treatment with the antagonist alone (Goldberg et al. 1981; Dykstra 1983; Schindler et al. 1990, 1993). In these experiments, antagonist-induced sensitization was only observed under conditions of repeated administration of high doses of antagonist in the absence of opiate agonist pretreatment. For example, Schindler and co-workers (1990, 1993) reported that weekly dosing with 100 mg/kg naltrexone using a cumulative dosing procedure resulted over the course of 8 weeks in a gradual shift to the left in the cumulative dose-effect function for naltrexone-induced suppression of operant response rates. However, it is unlikely that this phenomenon of antagonist-induced sensitization contributed significantly to the results of the present experiments, because naloxone is shorter acting than naltrexone, much lower doses of antagonist were employed, and no rat in the current study received more than three naloxone treatments.

As discussed previously, somatic withdrawal signs also can be precipitated by antagonists in a variety of animal species following a single opiate agonist pretreatment, typically under conditions of high doses of agonist and/or antagonist (Cheney and Goldstein 1971; Jacob and Michaud 1974; Jacob et al. 1974; Kosersky et al. 1974; Smits 1975; Wiley and Downs 1979; Ritzmann 1981; Krystal and Redmond 1983; Ramabadran 1983; Sofuoglu et al. 1990; Schnur et al. 1992) In seeming contrast, Adams and Holtzman (1990) noted that profound somatic indices of opiate withdrawal such as weight loss could not be precipitated by naltrexone even after prolonged periods of weekly pretreatment with moderate doses of morphine (3–5.6 mg/kg), under conditions in which the naltrexone dose-effect function for suppression of response rates shifted by 600-fold. Thus, despite a large body of literature, it remained unclear whether somatic signs of opiate withdrawal could be reliably observed following moderate doses of morphine and within the same dose range of antagonist that produced reliable suppression of operant responding. Moreover, it was unknown whether these somatic signs of withdrawal would increase in severity when naloxone was given following a second morphine exposure, as has been reported in humans (Azorlosa et al. 1994).

In that regard, the results of the current study clearly suggest that somatic signs of withdrawal can be elicited by low doses of naloxone (0.1–0.3 mg/kg) following a single morphine exposure, and these signs are more prevalent following a second morphine exposure (see Table 2). Indeed, the dose-response functions for naloxone effects on response rates and somatic withdrawal signs appeared to be similarly affected by repeated morphine exposure, with both measures yielding approximately a 5-fold increase in naloxone potency on Morphine Day 2 as compared to Morphine Day 1 according to a relative potency analysis (Tallarida and Murray 1986). In agreement with the report of Adams and Holtzman (1990), however, profound signs of opiate withdrawal such as weight loss, wet dog shakes, abdominal constrictions, ptosis, and diarrhea, commonly elicited by naloxone doses of 0.3–3.0 mg/kg in chronically-dependent animals (e.g. Wei et al. 1973; Gold et al. 1994; Schulteis et al. 1994), were rarely (if ever) observed in the current experiment even at the higher doses of naloxone. Nonetheless, the current data suggest that a somatic withdrawal syndrome, although much less intense than that observed in chronically dependent animals, can be elicited following single or repeated acute injections of morphine at doses of naloxone similar to those required to suppress operant response rates.

An additional point worth emphasizing regarding methodology employed in the current study is that the interval between morphine pretreatment and naloxone administration was held constant at 4 h. As described in the Materials and methods section, this interval was chosen based upon prior studies in rats (Young 1986; Adams and Holtzman 1990; White-Gbadebo and Holtzman 1994) which indicated that reliable increases in antagonist potency could be observed at this interval in the absence of detectable agonist (morphine 3.0–5.6 mg/kg) effects. Indeed, an inspection of the vehicle control data in Fig. 1 reveals that morphine pretreatment did not affect operant response rates 4 h post-morphine, even on Morphine Day 2, suggesting that acute agonist effects have dissipated at the time of naloxone challenge. Interestingly, Young (1986) reported that the sensitizing effect of a 10.0 mg/kg morphine pretreatment on naloxone-induced suppression of response rates was lost if the interval between morphine and naloxone administration was extended from 4 to 12 h. However, studies of acute dependence in human subjects have indicated that, depending on the dose of morphine, the ability of naloxone to elicit somatic signs of withdrawal could be detected at morphine-naloxone intervals up to 24 h (Heishman et al. 1989b, 1990; Kirby et al. 1990). Therefore, additional work with rats in which the interval between morphine and naloxone is varied systematically might prove valuable in elucidating further the optimal conditions for studying the acute dependence phenomenon with animal models.

In conclusion, the current study provides further evidence that adaptive changes can occur in response to a single injection of an opiate such as morphine, that these adaptive changes are further enhanced following a second morphine exposure, and that this latter phenomenon does not appear to be due entirely to conditioned changes in naloxone sensitivity. The ability to quantify the occurrence of these adaptive changes with indices that are well established as markers of withdrawal in chronically opiate dependent rats suggests that they may reflect the beginning stages in the development of a chronic opiate dependent state (see also Heishman et al. 1989; Adams and Holtzman 1990). Furthermore, the paradigm employed in the current study to demonstrate this acute adaptive state closely parallels that used in human studies of acute dependence (Jones 1980; Bickel et al. 1988; Heishman et al. 1989a,b, 1990; Kirby et al. 1990; Azorlosa et al. 1994). Therefore, this paradigm should be useful in determining the neural mechanisms mediating acute dependence, and in determining whether the mechanisms involved are similar to those mediating chronic opiate dependence.

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