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Acute dependence on, but not tolerance to, heroin and morphine as measured by respiratory effects in rhesus monkeys

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Abstract

Acute dependence on and tolerance to heroin and morphine were assessed in rhesus monkeys using measures of respiration. Respiratory frequency (f) and minute volume (V_e) were measured in monkeys breathing air or 5% CO_2 in air using a pressure-displacement plethysmograph. Cumulative doses of naltrexone (0.0001–1.0 mg/kg, i.m) did not alter these parameters in untreated monkeys. Twenty-four hours after a cumulative dose of heroin (1 mg/kg, i.m.), naltrexone produced an increase in both f and V_e when monkeys were breathing air or 5% CO_2. Following 1 to 3 days of treatment with heroin (0.5 mg/kg/day, i.m.) or morphine (16 mg/kg/day, i.m.), naltrexone produced an increase in f and V_e that was related to the dose of naltrexone and the number of days of agonist administration. Two days following termination of heroin administration, naltrexone-induced respiratory stimulation declined and had disappeared completely by the fifth day. In tolerance studies, heroin (0.032–0.5 mg/kg, i.m.) and morphine (1–16 mg/kg, i.m.) were injected cumulatively each day for three consecutive days. These drugs suppressed f and V_e to nearly the same extent on day 3 as they had on day 1 of administration. These results suggest that dependence to morphine and heroin can be measured under conditions of acute 1 to 3 day administration conditions in primates using f and V_e as reliable and quantitative indicators of opioid withdrawal. Under these conditions, tolerance does not occur.

Keywords: Respiration; Opioid; Tolerance; Dependence; (Rhesus monkey)

1. Introduction

The development of physiological dependence on opioids is revealed by the appearance of distinctive withdrawal signs following abrupt discontinuation of chronic opioid administration or by administration of an opioid receptor antagonist to subjects who have received opioids chronically. Precipitated withdrawal by antagonist administration can be demonstrated following fairly short-term administration of opioids and has been termed acute dependence (Martin and Eades, 1961, 1964). Acute dependence on morphine developed after a 4–8 h i.v. infusion in rats (Kishioka et al., 1995, 1996) and in dogs (Martin and Eades, 1964). A single injection of morphine to mice (100 mg/kg; Yano and Takemori, 1997), rats (10 mg/kg; Eisenberg, 1982), and humans (18 mg/70 kg; Heishman et al., 1990) was sufficient to produce antagonist-precipitated withdrawal signs.

Opioid withdrawal has been quantified in rodents using several different measures. Weight loss (Wei et al., 1973; Kishioka et al., 1994), plasma corticosterone increases (Kishioka et al., 1994), diarrhea, wet dog shakes, teeth chattering and spontaneous jumping (e.g., Kishioka et al., 1996; Bläsig et al., 1973) have yielded quantitative indicators of antagonist-precipitated withdrawal in rodents. In the rhesus monkey, some behavioral signs such as atypical position in cage, holding abdomen, vocalization, and dyspnea were observed during opioid withdrawal (Katz, 1986).

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These signs are difficult to quantitate. Changes in respiration could prove to be sensitive to opioid administration and withdrawal and easy to quantitate in non-human primates. The sensitivity to CO_2 in breathing air was increased during spontaneous morphine withdrawal in humans (Martin et al., 1968), and the respiratory rate parameter f was increased by administration of an opioid receptor antagonist to acutely morphine-treated dogs (Martin and Eades, 1961) and following chronic morphine in rhesus monkeys (Paronis and Woods, 1997a). In the current study, we determined whether acute physiological dependence developed during short-term (1 to 3 days) administration of heroin or morphine using measures of respiration as quantitative indicators of antagonist-precipitated withdrawal.

Several investigators have argued that tolerance to and dependence on opioids consistently develop together and that dependence is closely related to tolerance at a mechanistic level (e.g., Reisine and Pasternak, 1996). Findings that procedures that block development of tolerance to morphine may also block dependence (Abdelhamid et al., 1991; Trujillo and Akil, 1991) support this argument. Other evidence suggests that the two phenomena may be independent. Rahman et al. (1994), for example, demonstrated that pain-associated anxiety in mice induced by formalin administration delayed the development of tolerance to the analgesic effects of morphine as estimated by tail pinch, but did not modify the development of dependence as estimated by behavioral withdrawal signs. Moreover, Paronis and Woods (1997a,b) reported that daily administration of morphine (3.2 mg/kg) for over 43 weeks, produced no evidence of tolerance to the respiratory suppressant effects of morphine in rhesus monkeys, but did result in dependence on morphine. In the current study, in addition to measuring respiratory effects of naltrexone administration in acutely dependent monkeys, we also examined changes in the potency of heroin and morphine as indicators of tolerance in order to compare directly the development of tolerance and dependence on opioids under conditions of short-term administration.

2. Methods

2.1. Subjects

Six adult rhesus monkeys (*Macaca mulatta*), three males and three females, weighing between 4.5 and 11.0 kg were subjects in this study. They were housed individually in cages in a room with controlled temperature and light cycle (6:00 a.m.–6:00 p.m.). Water was available ad libitum, and the monkeys were fed approximately 30 biscuits (Purina Monkey Chow) daily, supplemented twice weekly with fresh fruit. The monkeys were trained to sit in primate-restraint chairs, and experimental sessions for each monkey were carried out typically no more frequently than once per week. The monkeys all had extensive histories with opioid administration, including administration of morphine, heroin, buprenorphine and a similar partial agonist, MC-CAM. At least 20 days elapsed between administration of these drugs and those of the current study. The procedures used in this study were approved by the University of Michigan Committee on the Use and Care of Laboratory Animals.

2.2. Apparatus

The apparatus used was similar to that described previously (Howell et al., 1988; France and Woods, 1990; Butelman et al., 1993). The monkey was seated in a restraint chair which was placed in a ventilated, sound-attenuating chamber. A helmet was placed over the monkey's head and sealed around its neck by two closely fitting latex shields. Gas (either air or a mixture of 5% CO_2 in air) flowed into the helmet and was pumped out at a rate of 8 liters/min. The monkeys' breathing produced changes in pressure inside the helmet that were measured with a pressure transducer connected to a polygraph (Grass Model 7); the data were recorded on a polygraph trace and in a microprocessor (IBM PCjr) via an analog-to-digital converter. The apparatus was calibrated routinely with known quantities of air.

2.3. Procedure

Experimental sessions consisted of several consecutive 30-min cycles, each consisting of 23 min of exposure to air followed by 7 min exposure to 5% CO_2 in air (hereafter referred to as 5% CO_2). Respiratory parameters were recorded continuously during sessions. Frequency (f), the number of inspirations per min, was directly determined. Minute volume (V_e), the number of mls of air inspired per min, was calculated from integration of the polygraph tracing. Tidal volume (V_t), the number of mls of air taken with each inspiration, was calculated as V_e/f. During the first few minutes of exposure to 5% CO_2, the animal's breathing rate increased; a stable rate of respiration in 5% CO_2 developed during the second 3 min of exposure to 5% CO_2. Data from the last 3 min of exposure to air and the second 3 min of exposure to 5% CO_2 of each cycle were used for the data analyses. During the first cycle, control respiratory values in air and 5% CO_2 were collected. Drug was administered intramuscularly during the first 3-min of the next and the subsequent three to four cycles.

Monkeys were divided into two groups. One group of three monkeys received heroin and the other three received morphine. In studies of acute dependence, these drugs were administered in a cumulative fashion with increasing

doses given during the initial 3 min of each 30-min cycle as described above. Cumulative doses of heroin were 0.032, 0.1, 0.32, and 0.5 mg/kg, and cumulative doses of morphine were 1.0, 3.2, 10, and 16 mg/kg. In initial studies, the maximum dose of heroin was 1.0 mg/kg and the maximum dose of morphine was 32 mg/kg. Some data from administration of these doses are described. However, there appeared to be a residual effect of these doses 24 h later, and accumulation over 3 days seemed possible. For the remainder of the studies, therefore, the largest dose of heroin was 0.5 mg/kg and the largest dose of morphine was 16 mg/kg.

In the determination of short-term acute dependence, heroin 0.1 mg/kg or morphine 3.2 mg/kg was given as a bolus injection followed 30 min later by the initial injection of cumulative doses of naltrexone to 1.0 mg/kg. In all other determinations of tolerance or acute dependence, the opioids were injected cumulatively (heroin to 0.5 mg/kg and morphine to 16 mg/kg) on either 1, 2, or 3 consecutive days, and naltrexone was given cumulatively in doses of 0.0001 to 1.0 mg/kg 24 h later. To measure recovery from acute opioid dependence, naltrexone was given 1 or 5 days following two consecutive days of heroin or morphine administration. Evaluation of the effects of different exposure periods was done separately, and at least 1 week was allowed between administration of the agonists. One exception to this was that only 2 days intervened between bolus administration of heroin 0.1 mg/kg followed 30 min later by cumulative naltrexone, and the study of tolerance to three consecutive days of heroin administration.

The studies of tolerance development were concurrent with the studies of acute dependence. The order of the heroin experiments was as follows: heroin 1 mg/kg on one session + naltrexone 24 h later; heroin 0.5 mg/kg/day for 2 days + naltrexone 24 h later; heroin 0.5 mg/kg/day for 3 days (tolerance evaluation) + naltrexone 24 h later; heroin 0.5 mg/kg on one session + naltrexone 24 h later; naltrexone alone; heroin 0.1 mg/kg bolus + naltrexone 30 min later; heroin 0.5 mg/kg/day for 3 days (tolerance evaluation) + naltrexone 24 h later; heroin 0.5 mg/kg bolus + naltrexone 30 min later; heroin 0.5 mg/kg/day for 2 days + naltrexone 5 days later. The order of the morphine experiments was morphine 32 mg/kg on one session + naltrexone 24 h later; morphine 16 mg/kg/day for 3 days (tolerance evaluation) + naltrexone 24 h later; morphine 16 mg/kg on one session + naltrexone 24 h later; naltrexone alone; morphine 3.2 mg/kg bolus + naltrexone 30 min later; morphine 16 mg/kg/day for 3 days (tolerance evaluation) + naltrexone 24 h later; morphine 16 mg/kg/day for 2 days + naltrexone 24 h later; morphine 16 mg/kg/day for 2 days + naltrexone 5 days later; morphine 16 mg/kg bolus + naltrexone 30 min later.

2.4. Drugs

The drugs used in these studies were heroin hydrochloride, morphine sulfate, and naltrexone hydrochloride (Research Technology Branch, National Institute on Drug Abuse, Rockville, MD). All drugs were dissolved in sterile water and injected i.m. in the side of the monkeys' thighs.

2.5. Data analysis

Values obtained in each experimental session are expressed as percent of a local control value. Control measures were taken in the first respiratory cycle, either prior to administration of the opioid receptor agonist or prior to administration of the opioid receptor antagonist. This differed depending on the experiments, and the control measures utilized are defined in the individual figure legends. Mean and S.E.M. values ($n = 3$) of the percent of control values were computed. Data were analyzed by a one-way analysis of variance followed by the Newman–Keuls test for multiple comparison and unpaired Student's *t*-test for comparison between two groups. Analyses of data were

Table 1
Respiration under control conditions (before opioid treatment) in six monkeys under air or 5% CO_2 conditions
Each value represents the mean ± S.E.M. of 10 experimental sessions.
Values in the parenthesis are expressed as percentage of values obtained with air.

Subject	Air			5% CO_2 in air		
	f (inspirations/min)	V_e (ml/min)	V_t (ml/inspirations)	f (inspirations/min; %)	V_e (ml/min; %)	V_t (ml/inspirations; %)
Heroin group						
CO	23.3 ± 0.8	2405 ± 170	102.9 ± 5.4	31.6 ± 1.0 (137.0 ± 6.3)	7352 ± 554 (309.0 ± 18.6)	231.2 ± 12.5 (225.5 ± 8.1)
EL	23.5 ± 0.8	2121 ± 178	87.7 ± 3.7	34.0 ± 1.4 (146.0 ± 7.6)	6471 ± 382 (318.8 ± 27.3)	189.7 ± 5.9 (217.8 ± 6.6)
SH	26.4 ± 1.3	2407 ± 151	91.6 ± 4.6	42.9 ± 1.6 (164.6 ± 6.6)	7474 ± 361 (315.8 ± 14.4)	176.5 ± 10.3 (192.5 ± 5.5)
Morphine group						
SA	24.9 ± 0.4	1521 ± 87	60.7 ± 2.9	35.8 ± 1.5 (143.6 ± 4.9)	5155 ± 333 (339.3 ± 11.9)	143.0 ± 4.4 (237.9 ± 7.6)
CL	31.6 ± 1.0	2539 ± 199	80.0 ± 5.0	45.6 ± 1.8 (144.3 ± 3.3)	7256 ± 457 (289.4 ± 9.7)	158.3 ± 5.8 (201.3 ± 7.4)
DA	31.7 ± 1.3	1783 ± 129	57.0 ± 4.4	42.6 ± 1.3 (135.3 ± 4.0)	6001 ± 482 (336.9 ± 13.3)	139.7 ± 8.3 (250.4 ± 10.9)

done using the computer program described by Tallarida and Murray (1987).

3. Results

3.1. Respiration under control conditions

Control ventilatory values, which were measured in the initial, pre-drug cycle in monkeys breathing air or 5% CO_2 are presented in Table 1 for the six subjects. These are averages of values taken in the initial, non-drug control cycle on each of 10 experimental sessions. These 10 pre-drug control measures were selected randomly from all of the pre-drug cycle control observations. The ranges of control values in monkeys breathing air were 23 to 32 inspirations/min for f, 1521 to 2539 ml/min for V_e, and 57 to 103 ml/inspiration for V_t. When monkeys were exposed to 5% CO_2, f increased to an average of 145% of control, V_e increased to an average of 318% of control, and V_t increased to an average of 221% of control. As shown in Table 1, two of the monkeys in the morphine group had substantially lower values of V_e and V_t than the

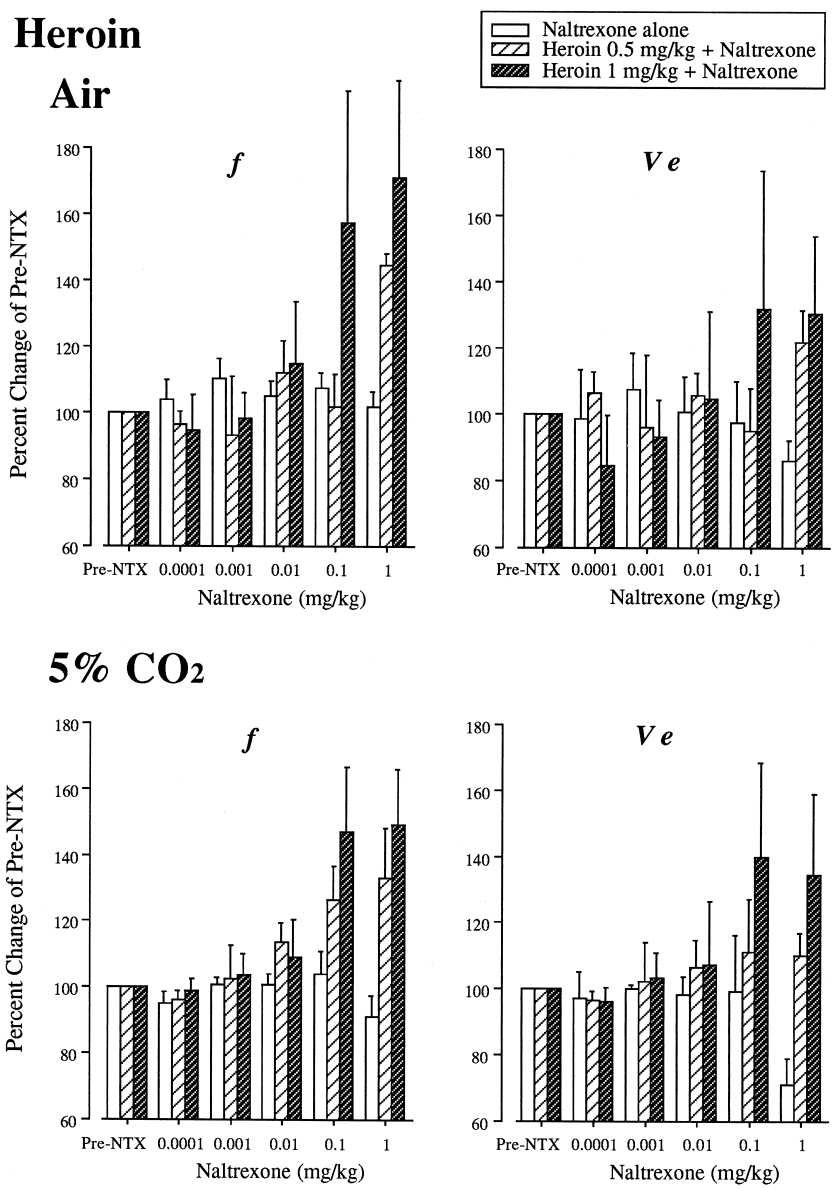

Fig. 1. Effects of cumulative naltrexone (NTX) on f (left panels) or Ve (right panels) in monkeys with no treatment (open bars) or treated with heroin 0.5 mg/kg (hatched bars) or 1.0 (solid bars) 24 h earlier. The effects of naltrexone when the monkeys were breathing air (top panels) and CO_2 (bottom panels) are shown. The control values are the respiration values obtained in the first cycle prior to administration of naltrexone, 24 h following heroin administration.

other monkey in this group or the monkeys in the heroin group. This was true under both conditions of breathing air and breathing 5% CO_2. There was no clear reason for these differences and they appeared to have little consequence for the effects of the opioids. Throughout the experiments, drug effects on f were more pronounced than effects on V_t or V_e; for this reason, data using f are emphasized in the graphical presentation.

3.2. Acute dependence

3.2.1. Effects of naltrexone alone and 24 h after opioid administration

Naltrexone, in doses of 0.0001–1.0 mg/kg did not cause marked changes in f and V_e when monkeys were breathing air or 5% CO_2. The effects of naltrexone given alone or 24 h after a total cumulative dose of 0.5 or 1 mg/kg heroin are shown in Fig. 1. When calculated as a percent change from the baseline values, naltrexone produced increases in respiration that were not statistically significant 24 h following a single injection of heroin. The data suggest as well that when the larger dose of heroin was given the previous day, smaller doses of naltrexone were needed to increase f. There was also an indication that f was more influenced by naltrexone following heroin administration than was V_e. Very similar effects of naltrexone were shown 24 h following administration of 16 and 32 mg/kg morphine (data not shown). Because the effects of heroin–naltrexone combinations were the same when the monkeys were breathing air as when they were breathing 5% CO_2, only the effects on 5% CO_2 are shown in the following figures.

3.2.2. Effects of naltrexone following multiple days of heroin or morphine administration

Fig. 2 (left panel) shows the results of cumulative naltrexone administration to monkeys 24 h following either 1, 2, or 3 days of administration of 0.5 mg/kg heroin. As was shown above, following a single administration of 0.5 mg/kg heroin, the increase in f produced by naltrexone 1.0 mg/kg was not significant. Following two or three consecutive days of heroin administration, both 0.1 and 1.0 mg/kg naltrexone produced significant increases in f.

Comparable results were observed after pretreatment with morphine (16 mg/kg/day) for 1–3 days (Fig. 2, right panel). Significant increases in f were produced by the largest dose of naltrexone after 2 or 3 days of daily morphine administration.

3.2.3. Loss of sensitivity to naltrexone

As described above, 24 h following the second of two consecutive days of heroin 0.5 mg/kg or morphine 16 mg/kg administration, naltrexone 1.0 mg/kg produced increases in f and V_e. When naltrexone was given 5 days

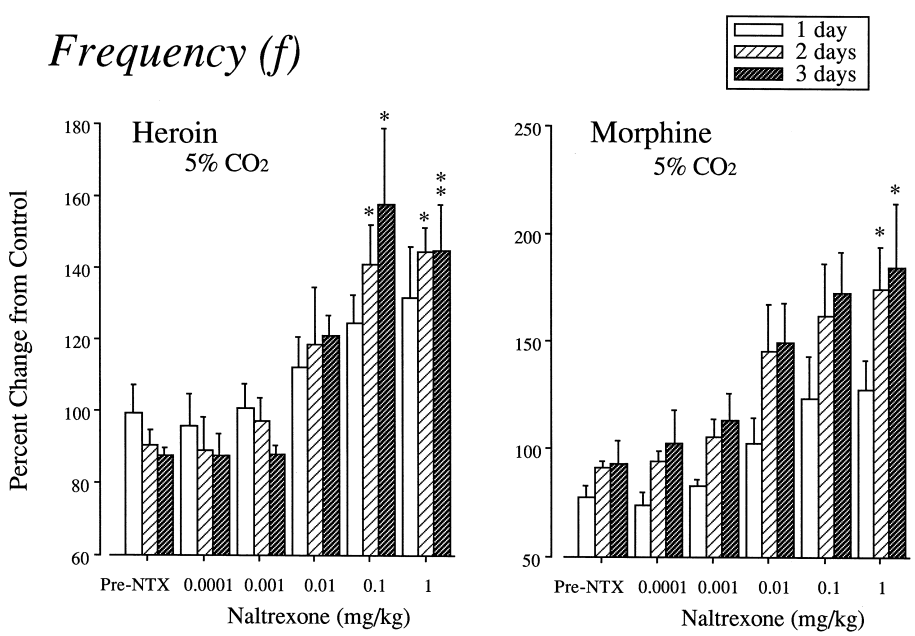

Fig. 2. The effects of cumulative naltrexone (NTX) on f in monkeys who received heroin 0.5 mg/kg (left panel) or morphine 16 mg/kg (right panel) for one day (open bars), or for two (hatched bars) or three (solid bars) consecutive days. Values in the 5% CO_2 condition are shown. The control value is f measured in the first cycle prior to opioid receptor agonist administration on the first day. The Pre-NTX values are taken in the first cycle prior to naltrexone administration. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

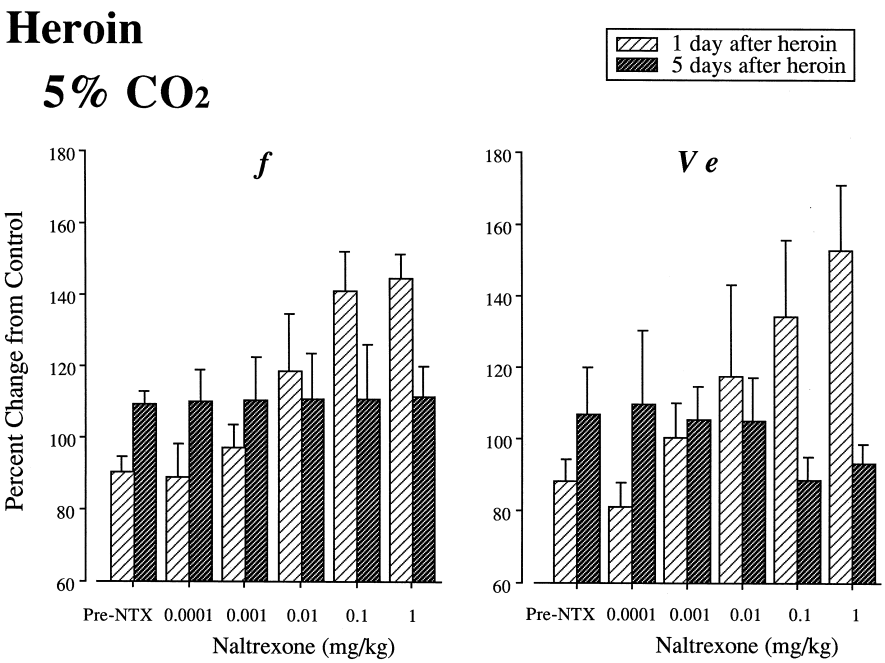

Fig. 3. Recovery of sensitivity of f (left panel) and V_e (right panel) in 5% CO_2 to cumulative naltrexone (NTX) administration. Heroin 0.5 mg/kg was given cumulatively on one session and NTX was given cumulatively 24 h later (hatched bars), or heroin 0.5 mg/kg was given cumulatively on two consecutive days and NTX was given 5 days after the second day of heroin administration (solid bars). The control baseline are rate data taken on the first cycle prior to the first day (or only day) of heroin administration. The Pre-NTX control data are taken from the first cycle prior to administration of cumulative NTX.

following the second of two consecutive days of heroin 0.5 mg/kg administration, it had no effect on any of the respiratory parameters (Fig. 3). The pre-naltrexone baseline values for f and V_e were slightly decreased one day following heroin administration, perhaps reflecting a residual effect of heroin administration, and slightly increased 5

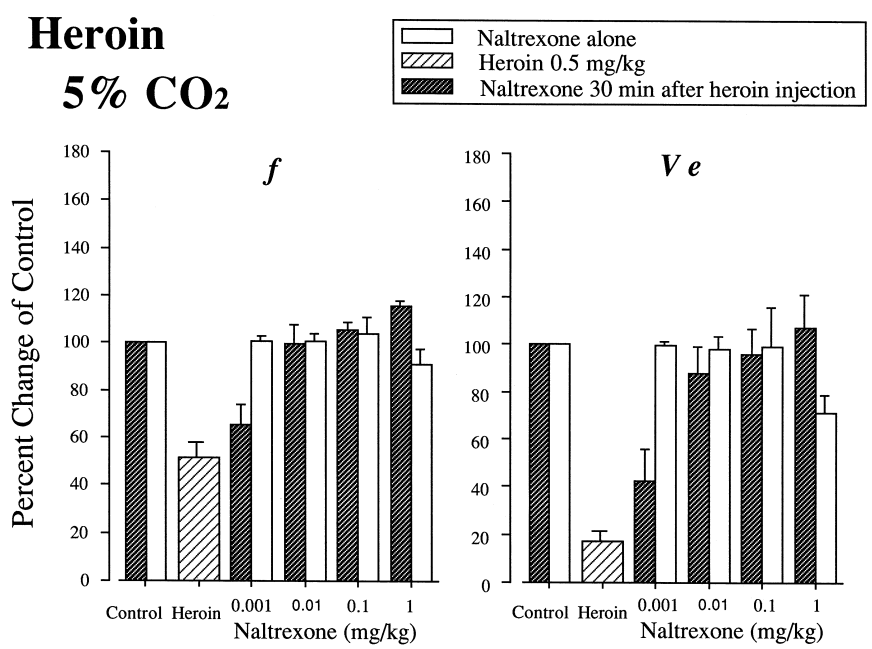

Fig. 4. The effects of cumulative naltrexone on f (left panel) and Ve (right panel) in 5% CO_2 when given alone (open bars) or 30 min following a bolus injection of heroin 0.5 mg/kg (closed bars). The hatched bar is the effect of the bolus injection of heroin 0.5 mg/kg prior to administration of naltrexone. The control values are taken in the first cycle prior to administration of naltrexone alone (open bars) or prior to administration of the bolus injection of heroin.

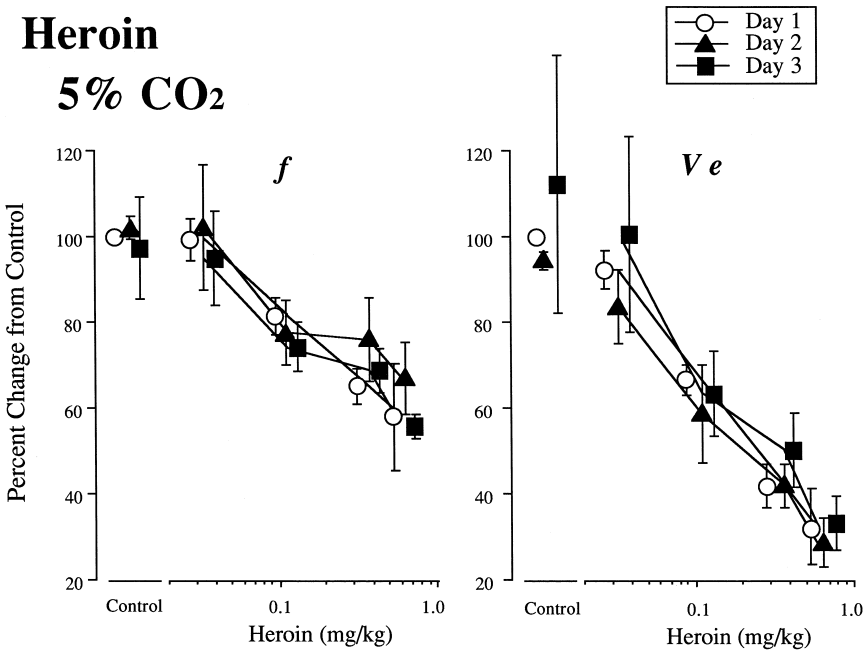

Fig. 5. The effects of repeated cumulative administration of heroin on f (left panel) or Ve (right panel) in monkeys breathing 5% CO_2. Heroin was given for one (open circles), two (closed triangles), or three (closed squares) consecutive days in separate experiments. The control axis is f (left panel) or V_e (right panel) taken during the first cycle, prior to the first cumulative administration of heroin. The control points are either this same control value (open circle) or the respiration data taken on the first cycle prior to the second (closed triangle) or third (closed square) cumulative administration of heroin.

days following heroin administration. This led to a difference between the 1 and 5 post-heroin day baseline values. When naltrexone was given five days after the second of two consecutive days of morphine 16 mg/kg, it also failed to elicit any change in respiratory parameters (data not shown).

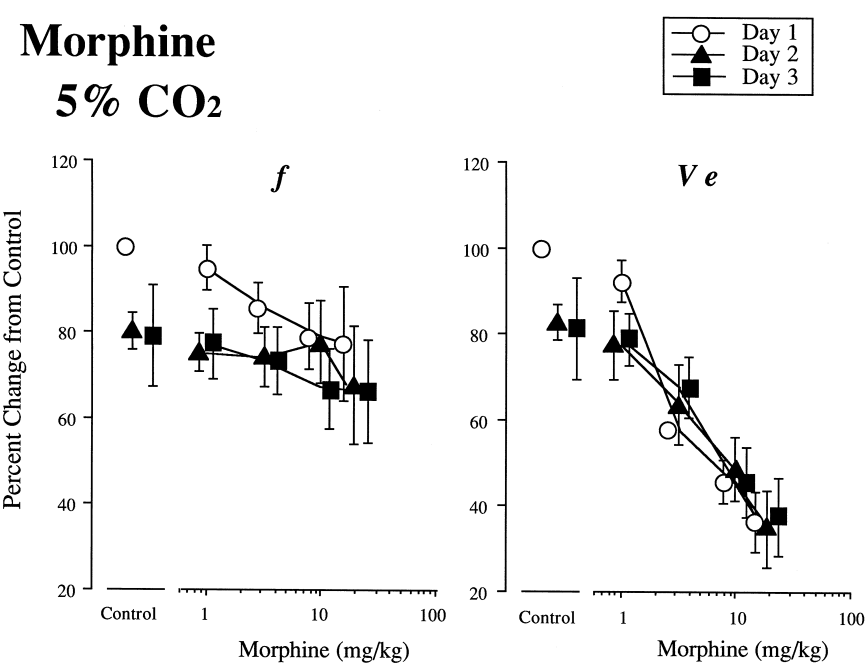

Fig. 6. The effects of repeated cumulative administration of morphine on f (left panel) or V_e (right panel) in monkeys breathing 5% CO_2. Morphine was given for one (open circles), two (closed triangles), or three (closed squares) consecutive days in separate experiments. The control axis is f (left panel) or V_e (right panel) taken during the first cycle, prior to the first cumulative administration of morphine. The control points are either this same control value (open circle) or the respiration data taken on the first cycle prior to the second (closed triangle) or third (closed square) cumulative administration of morphine.

3.2.4. Effects of naltrexone 30 min after a single injection of heroin

Heroin 0.5 mg/kg given acutely produced 48% and 83% decreases in f and V_e, respectively. Administration of cumulative doses of naltrexone 30 min following administration of heroin 0.5 mg/kg resulted in an attenuation of the respiratory suppressant effects of heroin, but did not increase these measures over baseline values (Fig. 4). Similar results were found when naltrexone was given 30 min following acute administration of morphine 16 mg/kg (data not shown).

3.3. Tolerance

3.3.1. Effects of repeated administration of heroin or morphine

On the first day of cumulative heroin administration (0.032–0.5 mg/kg), f and V_e in 5% CO_2 were suppressed by heroin in a dose-dependent manner (Fig. 5). The dose–response curves of the effects of heroin on f and V_e on the second and third days virtually overlapped those obtained on day one.

The effects of one, two, and three days of morphine administration on f and V_e are shown in Fig. 6. Although the effects of morphine on f appeared to increase somewhat from day one to day two, this was not significant, and, perhaps more important, this effect was not further enhanced by another day of morphine administration. Measures of V_e were unchanged during this time.

4. Discussion

Twenty-four hours after a cumulative injection of heroin 0.5 mg/kg, naltrexone elicited a dose-dependent, non-significant increment in f. Twenty-four hours following a 2-day regimen of heroin 0.5 mg/kg or morphine 16 mg/kg administration, the effects of naltrexone 1.0 mg/kg on f were more marked and statistically significant. Naltrexone (0.0001–1.0 mg/kg) by itself did not affect the respiratory parameters, consistent with earlier results of Paronis and Woods (1997a). These results suggest that dependence had begun to develop following single injections of the μ-opioid receptor agonists and was clearly present after two such injections, 24-h apart. Rate of respiration has been used in other studies as an indicator of opioid withdrawal in primates. Respiration rate was increased during both naltrexone-precipitated and spontaneous withdrawal in rhesus monkeys (Paronis and Woods, 1997a), and sensitivity to CO_2 effects on respiration rate was increased during spontaneous morphine withdrawal in humans (Martin et al., 1968). These data are consistent with those of Heishman et al. (1990) who observed naloxone-precipitated withdrawal 24 h after a single injection of morphine 18 mg/70 kg, i.m. in humans, using subjective symptoms and observer-rated signs of opioid abstinence. Azolosa et al. (1994) also found in humans that two injections of morphine, 24-h apart, resulted in more intense precipitated withdrawal following naloxone administration than did a single morphine injection. Thus, respiration appears to be a sensitive measure of opioid withdrawal in the non-human primate, and reflects the development of acute dependence to opioid receptor agonists that parallels data found using other measures in humans.

Naltrexone induced-respiratory stimulation had dissipated by 5 days after discontinuance of heroin or morphine administration indicating that the changes brought about by the 2-day exposure to opioid receptor agonists had declined during these 5 days. In addition, our results showed that whereas naltrexone administration 24 h after opioid treatment produced an increase in f, if the heroin or morphine was given only 30 min prior to naltrexone, the antagonist simply reversed the respiratory depressant effect of heroin. No rebound increase in f was observed. Thus, with the doses of naltrexone used here, a certain amount of time, something between 30 min and 24 to 48 h, was required following heroin administration before naltrexone elicited increased f. During this time it appears that dependence begins to develop, and over the course of 5 days following cessation of agonist administration, this acute dependence has dissipated. Eisenberg (1982) reported that naloxone was able to elicit signs of the acute morphine dependence as soon as 30 min following agonist administration, using an increase in plasma corticosterone as a measure. June et al. (1995) found in humans that the naloxone-precipitated withdrawal signs peaked 6 h following administration of a single dose of 18 mg/70 kg morphine and had declined by 42 h. We did not evaluate acute dependence at these time periods, but our data support the notion that some time is required for morphine dependence to develop, and that this phenomenon is time-limited.

In the presence of normal air, f increased 171.2 ± 29.5% when naltrexone 1 mg/kg was given 24 h following administration of heroin 1 mg/kg and the corresponding V_e increased 130.4 ± 23.6%. In the presence of 5% CO_2, f increased 149.3 ± 16.8% and V_e increased 134.6 ± 24.5% following this combination of naltrexone and heroin. These data indicate that naltrexone-induced increments in f and V_e were similar whether the monkeys were breathing air or 5% CO_2 suggesting that the heroin withdrawal-induced increase in respiration were independent of the ambient concentrations of CO_2.

These experiments demonstrate that the magnitude of the increases in f and V_e is dependent on (1) the dose of naltrexone, (2) the dose of the opioid receptor agonist, and (3) the number of days of heroin or morphine administration. This is consistent with the results of Kishioka et al. (1994, 1996) who showed that naloxone-induced body weight loss, diarrhea, and plasma corticosterone increase in rats given morphine were related to each of these three factors.

Heroin and morphine suppressed the f and V_e in a dose-dependent manner, and the potency of these agonists in suppressing these respiratory parameters was not altered during three days of administration. Thus, tolerance to these opioid receptor agonists was not observed during the same time and dose parameters over which acute dependence was observed. Ling et al. (1989) reported that the development of tolerance to the respiratory depressant effects of morphine in rats was not observed following an i.v. infusion of morphine 50 μg/kg/hr for 10 h, whereas tolerance to morphine analgesia did develop under this regimen. Paronis and Woods (1997b) showed that the tolerance to morphine-induced respiratory suppression did not develop after 43 weeks of daily administration of morphine (3.2 mg/kg/day) in rhesus monkeys, even though tolerance to morphine analgesia developed over this time period. Thus, the current study supports earlier studies that demonstrated that under some conditions of administration, there is little tolerance to the respiratory depressant effects of opioids.

Tolerance to the respiratory depressant effects of morphine and fentanyl in rodents has been reported following morphine pellet implantation (Bowen et al., 1979; Roerig et al., 1987) and by fentanyl s.c. infusion using an osmotic minipump (Ayesta and Florez, 1990). However, McGilliard and Takemori (1978) showed that tolerance to respiratory depression did not develop following a single injection of morphine (20 mg/kg s.c.) 6 h before testing, although it did develop following morphine pellet implantation. Therefore, the lack of development of tolerance to heroin- and morphine-induced respiratory depression in the current experiment may be due to the regimen of opioid administration (i.e., once daily for 3 days vs. chronic morphine exposure produced by pellet implantation or chroinc s.c. infusion). The reasons why continuous exposure of the mu receptor to an agonist is important in the development of tolerance to the respiratory effects of these drugs is not clear. It is also not known whether chronic infusions of opioids would produce tolerance to respiratory effects in monkeys. Certainly, extremely long periods of daily morphine injections did not result to tolerance to the respiratory effects of morphine in rhesus monkeys (Paronis and Woods, 1997b).

In conclusion, we have demonstrated that dependence development to heroin or morphine can occur with very short-term exposure to these opioids. The respiratory parameter f is a reliable and quantitative indicator of acute opioid dependence. Tolerance to the respiratory depressant effects of heroin and morphine was not observed under these experimental conditions.

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