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Heroin Self-Administration by Rats: Influence of Dose and Physical Dependence

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DAI, S., W. A. CORRIGALL, K. M. COEN AND H. KALANT. *Heroin self-administration by rats: Influence of dose and physical dependence*. PHARMACOL BIOCHEM BEHAV **32**(4) 1009–1015, 1989.—Lever-pressing behavior reinforced by intravenous infusion of various concentrations of heroin, and consequent development of physical dependence, were examined in rats. In addition, the influence of opiate dependence, and of its disappearance following withdrawal, on heroin self-administration were investigated. It was found that intravenous self-administration of heroin at 0.03 mg/kg/infusion maintained self-administration behavior without producing physical dependence. Total responses per session decreased with increasing unit dose of heroin, whereas the total amount of drug self-administered was directly related to unit dose. Significantly greater numbers of withdrawal signs and percentage body weight losses in response to naloxone injections were observed following self-administration of heroin at 0.1, 0.3 or 0.6 mg/kg/infusion. Intake of heroin at 0.03 mg/kg/infusion, but not at 0.1, 0.3 or 0.6 mg/kg/infusion, was found to increase significantly in opiate-dependent and postdependent animals. These findings support the previous use of 0.03 mg/kg/infusion as a suitable dose for illustrating the reinforcing effect of heroin without the influence of physical dependence.

Heroin Intravenous self-administration Physical dependence Dose Reinforcement Rat

THE technique of self-administration in experimental animals allows determination of whether a compound has reinforcing efficacy. Reinforcing efficacy in animals has proven to be highly correlated with dependence potential in humans (23). Many studies have demonstrated that rats self-administer morphine and other opiates intravenously on a sustained basis (16,17) and in dose-dependent fashion. Although the reinforcing property of opiates may occur without behavioral evidence of physical dependence, self-administration of high concentrations of morphine has been shown to produce withdrawal symptoms when the drug was no longer available (21).

Intravenous (IV) self-administration of heroin has recently been used to investigate the mechanisms of, and the sites in the central nervous system involved in, opiate reinforcement [e.g., (3, 5, 6, 18, 20)]. These studies tacitly assume that IV self-administration of low concentrations of heroin does not induce physical dependence. However, because no thorough study has been reported on the relationship between the development of physical dependence and the dose of intravenously self-administered heroin, there is no firm evidence to support this assumption.

In the present study, we have examined the development of physical dependence in rats following IV self-administration of various concentrations of heroin. The objective was to determine the minimum concentration of heroin which would induce physical dependence in limited-access sessions, and to assess the severity of physical dependence in relation to the dose of infused opiate. We have also investigated the influence of physical dependence, and of the postdependent state, on drug-reinforced responding.

METHOD

General

Male Long-Evans rats (Charles River, Lachine, Quebec), weighing 260–300 g, were used. They were singly caged and maintained on a reversed light-dark cycle (lights off between 7:00 and 19:00 hr), initially with food and water available ad lib for several weeks.

Training Procedure and Implantation of IV Catheters

The animals were food restricted and trained to respond on a continuous reinforcement (CRF) schedule for 45-mg food pellets. Once so trained, they were maintained on 4 pellets (approximately 20 g) of standard laboratory chow daily for the duration of the experiment. This amount of food constitutes the recommended daily nutritional requirement for the rat, and animals in this study

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were not maintained in a food-deprived state, although they were obviously hungry at the time of the experiment. Over the course of this study, animals gained an average of 25 g body weight. Although this is a smaller gain than that resulting from ad lib feeding, the animals remained in excellent health throughout the experiment.

Each rat was then surgically prepared with a chronic IV catheter under general anesthesia with acepromazine maleate (Ayerst; 10 mg/kg IP) and ketamine HCl (Rogar STB; 100 mg/kg IM). The catheter was inserted into the right jugular vein and exteriorized between the scapulae. Animals were allowed to recover for 4–7 days before drug self-administration sessions were begun.

Drug Self-Administration

Drug self-administration sessions were carried out in dual-lever operant chambers. Responding on one lever (active lever) led to drug delivery via the IV catheter under a CRF schedule (100 μl/kg; one second infusion time). Responding on the other lever (inactive lever) had no consequences for the subject but was recorded as a measure of lever-pressing behavior not paired with drug delivery. Each drug delivery was followed by a 1-min time-out period, signalled by a light and tone combination, during which responding on the active lever was also recorded but not reinforced.

Animals were initially given access to cocaine (1 mg/kg/infusion) in 90-min sessions under a CRF schedule for 5 days, to ensure that acquisition of drug self-administration had occurred prior to opiate exposure and withdrawal testing. For self-administration sessions after cocaine, sessions were 30 min each and occurred twice each weekday at approximately 9:30 and 15:30 hours. The animals were given access to saline during the first week after cocaine self-administration, in order to obtain baseline values for withdrawal measures and to extinguish cocaine-reinforced responding. Starting with the second week postcocaine, animals had access to increasing concentrations of heroin solution each week such that unit doses in successive weeks were 0.03, 0.1, 0.3 and 0.6 mg/kg/infusion. Each dosage level was maintained for one week, and then replaced by the next higher. Weekends (Saturday and Sunday) were drug-free for all subjects.

Examination for the Development of Physical Dependence

Rats were examined for the presence of opiate dependence after the morning session of the fifth day at each dose, including saline (i.e., after the 9th session, at approximately 11:00–11:30 hr on the last day of each week). Physical dependence on heroin was evaluated by scoring the withdrawal syndrome precipitated by intraperitoneal injection of 1 mg/kg naloxone HCl (4,12). Immediately after naloxone administration, each animal was placed individually in its own cage, and the following behavioral signs were monitored for 20 min: wet-dog shakes, head shakes, ptosis, teeth chattering, writhing, chewing, paw tremor and diarrhea. The body weight loss at 4 hour after naloxone injection was also measured. No food or fluid was given during this 4-hour period. The animals were allowed to self-administer their afternoon doses of heroin, or saline, after these measurements were completed.

Heroin Self-Administration in Opiate-Dependent Rats

Animals which had self-administered heroin at the 0.6 mg/kg/infusion dose (and were found to be opiate-dependent) were not given access to heroin over the following weekend. However, to facilitate maintenance of their opiate dependence, they were given access to 0.6 mg/kg/infusion heroin during the following Monday self-administration session. They were subsequently tested in the self-administration paradigm at each of the previous unit doses, one day (i.e., two sessions) at each dose.

Opiate Withdrawal

Following the above test, animals were given access only to saline to self-administer. They were examined for the presence or absence of a naloxone-precipitated withdrawal syndrome on the 3rd and 7th day after the last heroin self-administration session.

Heroin Self-Administration in Postdependent Rats

Three days after the rats were found to be free of opiate dependence, they were again given access to IV heroin at increasing unit doses (0.03, 0.1, 0.3 and 0.6 mg/kg/infusion), one day (2 sessions) at each dose.

Drugs

The drugs used were as follows: diacetylmorphine hydrochloride (F. E. Cornell), cocaine hydrochloride (BDH), and naloxone hydrochloride (E. I. Du Pont de Nemours). All doses are expressed as the salt.

Analysis

For data presentation, number of responses or lever presses refers to the total count on a given lever; for the active lever, therefore, responding refers to the sum of drug-reinforced counts and those occurring in the time-out periods.

The data were expressed as the mean ± standard error of the mean (SEM), and were analyzed statistically by means of analysis of variance (ANOVA) with repeated measures, using post hoc tests (Duncan Multiple Range Test) as required. To compare intake of heroin in animals during initial exposure, average values over each week were used; values on the 9th session were used to compare responding at different doses during initial exposure. To compare responding and drug intake across initial exposure, dependent and postdependent states, data from the last sessions at each state were used. Therefore, values for initial exposure from the 9th session at each dose were compared with values from the 2nd session for dependent and postdependent animals. (The ninth session for initial exposure was used since it was the last session prior to dependence testing. Because dependent and postdependent animals were given access to each dose for a single day, the second session values were used.)

Data reported are from 8 animals which completed all treatments.

RESULTS

IV Heroin Self-Administration

Figure 1a shows the average daily IV heroin intake in these animals at the various unit doses tested. As the dose of the infused opiate increased, the daily intake increased in proportion to the dose. A single factor repeated measures ANOVA showed that there was a highly significant effect of unit dose on drug intake, $F(3,21) = 88.90$, $p < 0.001$.

For comparison, average values for active and inactive lever presses in the 9th session of self-administration of saline or heroin (prior to the naloxone-precipitated withdrawal test) are shown in Fig. 1b. There was a dose-dependent decrease in responding on

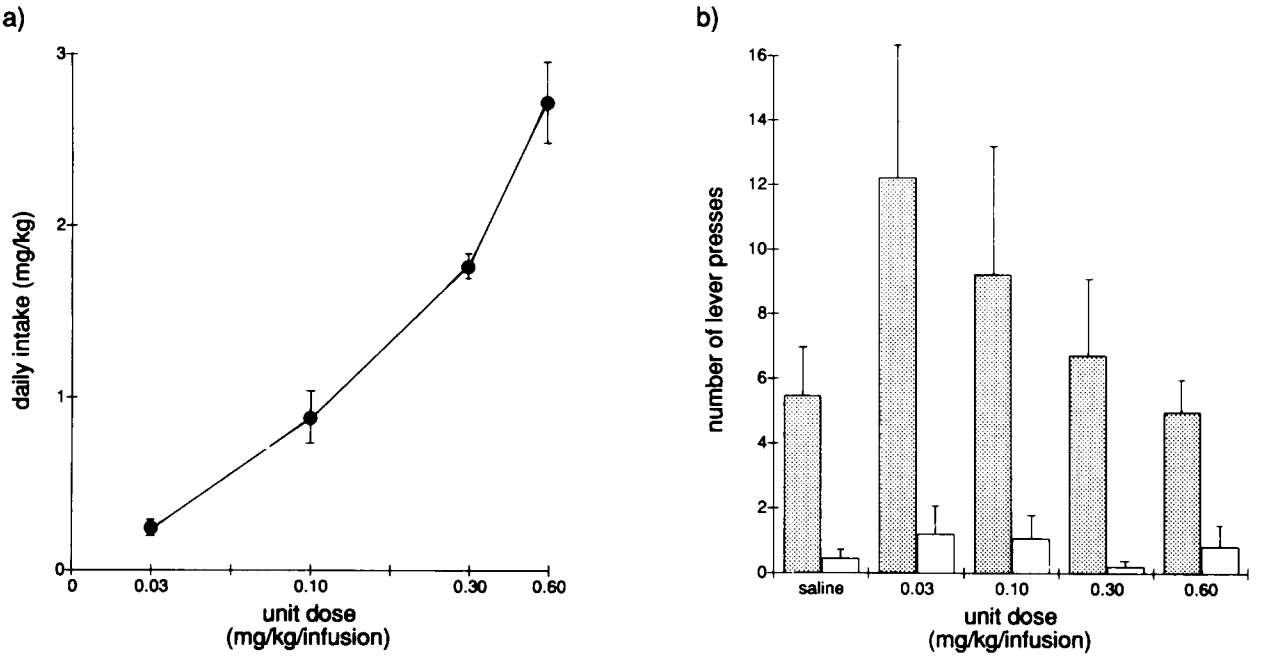

FIG. 1. (a). Average daily heroin intake during self-administration sessions. Each unit dose was available for 5 days. In this and subsequent figures, data are presented as mean ± SEM (standard error of the mean), for a sample of 8 subjects. (b) Average numbers of presses on active and inactive levers on the 9th self-administration session at each unit dose of heroin, including saline.

the drug-reinforced lever as the unit dose of heroin increased, but the effect was not statistically significant, $F(3,21) = 1.31$, $p > 0.20$. Responding on the inactive lever was significantly less than responding on the drug-reinforced lever, $F(1,7) = 20.92$, $p > 0.005$.

Naloxone-Precipitated Withdrawal Syndrome

Figure 2 shows the quantitative assessment of the naloxone-precipitated withdrawal syndrome in rats receiving IV saline or heroin. Inspection of the data shows that as the unit dose of heroin was increased, both withdrawal signs (Fig. 2a) and body weight loss (Fig. 2b) increased. For each measure, analysis of variance of the data between 0 (saline) and 0.60 mg/kg/infusion showed a significant effect of dose [for withdrawal signs, $F(4,28) = 37.85$, $p < 0.001$; for body weight loss, $F(4,28) = 5.01$, $p < 0.005$]. Post hoc tests showed that withdrawal signs after 0.1, 0.3 and 0.6 mg/kg/infusion were significantly greater than after saline or 0.03 mg/kg/infusion ($p < 0.01$). In addition, there were significantly fewer withdrawal signs at 0.10 than at 0.30 mg/kg ($p < 0.05$). For weight loss, post hoc tests showed that the dose of 0.03 mg/kg/infusion led to significantly smaller losses than 0.10 mg/kg/infusion ($p < 0.05$) or 0.30 and 0.60 mg/kg/infusion ($p < 0.01$); doses of 0.30 and 0.60 mg/kg/infusion produced significantly greater weight loss than saline ($p < 0.05$).

Naloxone induced fewer withdrawal signs on the third day following heroin withdrawal, but still more than control values. However,the percentage body weight loss at 4 hr after naloxone on the third day of saline substitution was similar to that in saline controls prior to heroin exposure. By the seventh day following heroin withdrawal, the number of withdrawal signs had returned to saline control values. Analysis of variance of the data at 0.60 mg/kg/infusion, 3, and 7 days postopiate showed that there was a significant decrease in withdrawal signs, $F(2,14) = 21.85$, $p < 0.001$, and weight loss, $F(2,14) = 9.42$, $p < 0.005$, between the last day of heroin access and 7 days later.

Of the eight withdrawal signs that were scored, ptosis, writhing, paw tremor and teeth chattering showed the most prominent dose-related increases during self-administration. Head shakes were observed only after the highest doses of heroin, and only in 1–2 subjects. The prevalence of wet dog shakes did not increase at higher self-administration doses. Scores for diarrhea and chewing behavior are not shown in Fig. 3. The former were not included because none of the animals exhibited diarrhea after any of the heroin doses; in the case of chewing behavior, scores were high even before heroin access, and they remained high throughout all doses of heroin.

IV Heroin Self-Administration in Opiate-Dependent and Postdependent Rats

The numbers of presses on the active lever at each dose during the initial exposure, after development of physical dependence, and in the postdependent state, are shown in Fig. 4. In the initial-dose exposure, there were relatively small changes in active-lever responses when animals received higher unit doses (0.1, 0.3 and 0.6 mg/kg/infusion) of heroin. During IV self-administration of heroin at 0.03 mg/kg/infusion, animals in opiate-dependent and postdependent states exhibited greater numbers of active-lever presses than in the initial exposure. At the higher unit doses, on the other hand, the dependent and postdependent rats showed substantial progressive decreases in responding on the active lever, relative to the high values at 0.03 mg/kg/infusion. Analysis of variance with two factors (condition: initial exposure, dependent and postdependent; dose: 0.03, 0.01, 0.30, and 0.60 mg/kg/infusion) showed a significant main effect of dose, $F(3,21) = 11.15$, $p < 0.001$, and a significant condition ×

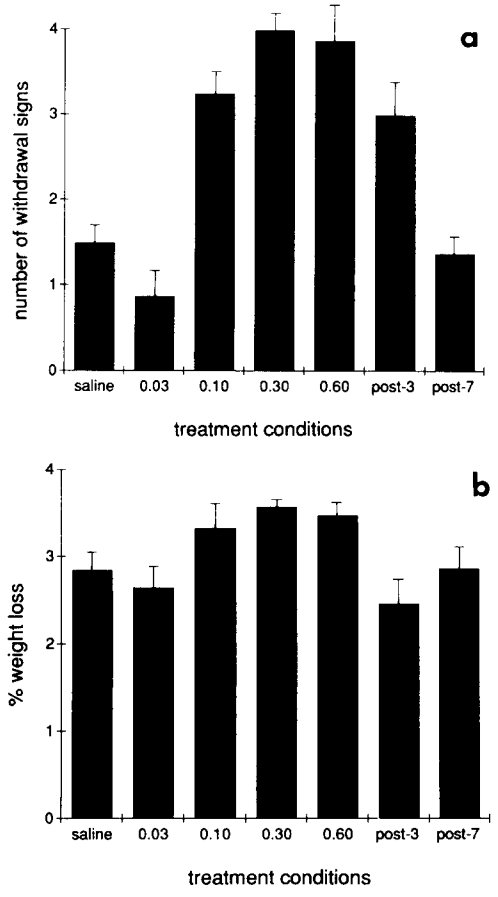

FIG. 2. Average number of withdrawal signs (a) and percent body weight loss (b) on tests for naloxone-precipitated withdrawal before, during and after heroin self-administration. The values of 0.03, 0.10, 0.30 and 0.60 on the abscissa refer to the unit dose of heroin; post-3 and post-7 refer to values at 3 and 7 days, respectively, after the last day of heroin self-administration.

dose interaction, $F(6,42)=3.00$, $p<0.05$. There was, however, no significant effect of condition, $F(2,14)=1.79$, $p>0.20$.

Figure 5 shows the session-average heroin intake over the three conditions at each dose. At a heroin dose of 0.03 mg/kg/infusion, both opiate-dependent and postdependent rats had higher heroin intakes than during the initial-dose exposure. There was a progressive increase in heroin intake in all groups when higher unit doses of the drug were available, and the differences between the three conditions varied at higher doses. Analysis of variance did not show any significant effect of condition, $F(2,14)=1.51$, $p>0.25$, only of dose, $F(3,21)=93.33$, $p<0.001$.

In view of the fact that unit doses of 0.01 mg/kg/infusion and higher produced physical dependence during initial exposure, differences between dependent and initial exposure conditions would not be expected at doses greater than 0.1 mg/kg for either responses or intake. Analysis of variance of the intake data and the response data for the three conditions at only the 0.03 mg/kg unit dose showed significant effects of condition [for response data, $F(2,14)=5.76$, $p<0.05$; for intake data, $F(2,14)=11.40$, $p<0.005$]. Post hoc tests for each of responding and intake showed that values during initial exposure were significantly different from those in both dependent ($p<0.01$) and postdependent animals ($p<0.05$); in addition, both intake and responding of postdependent animals were significantly less than values for dependent subjects ($p<0.05$).

DISCUSSION

It has been reported that when rats are allowed to self-administer morphine sulphate 0.03–10 mg/kg/infusion (IV), the number of infusions decrease with increasing unit dose while the amount of drug self-administered is directly related to unit dose (14). Similar, but not identical, findings were observed in the present study. In our study, the reinforcing property of heroin was illustrated by the fact that responding for heroin was greater than for saline at low unit doses, and by the greater number of active versus inactive lever presses. As larger unit doses were available, numbers of active lever responses decreased, but the daily heroin intake increased progressively. The latter finding appears to argue

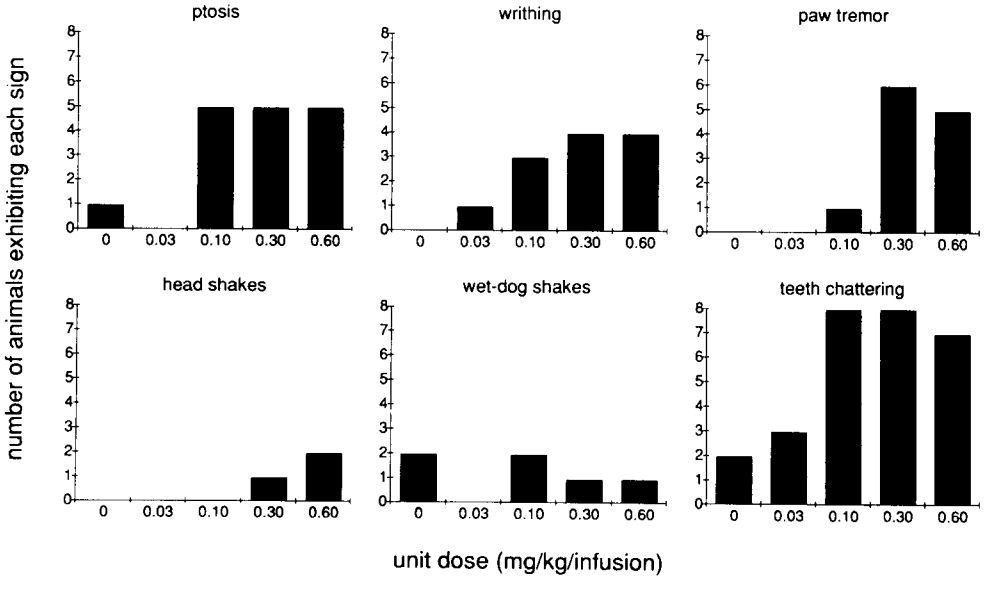

FIG. 3. Numbers of animals showing each of six of the withdrawal signs that were scored.

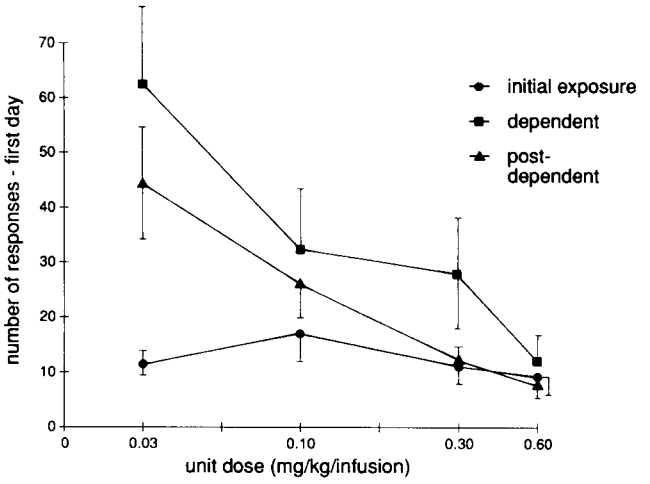

FIG. 4. Response on the drug-reinforced lever during conditions of initial exposure, opiate dependence, and postdependence. For initial exposure, values are from the 9th session; for opiate dependence and postdependence, values are from the 2nd session.

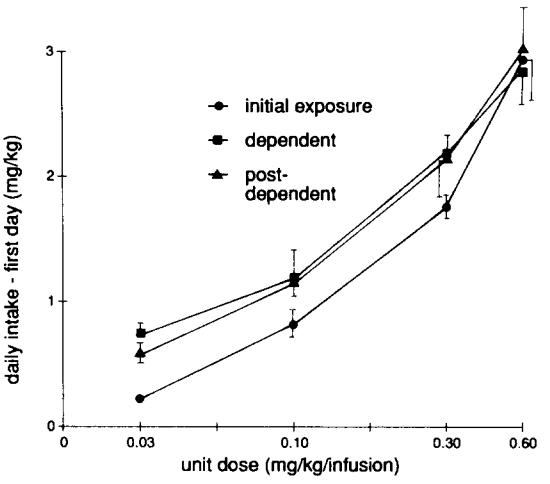

FIG. 5. Average heroin intake in a session at each unit dose during initial exposure, dependent, and postdependent conditions. As in Fig. 4, values for initial exposure are from the 9th session; for opiate dependence and postdependence, values are from the 2nd session.

against a satiation effect, and suggests that the decreased number of responses may represent the sedative effect of heroin. In any case, the 0.03 mg/kg unit dose seems to be more appropriate than the higher doses for demonstrating the reinforcing effect in the absence of abstinence effects.

Saline response rates in this study were high; during the 9th session of saline, infusions were still as high as 5–6/30 min. This is likely due to animals having had access to cocaine during the week prior to saline self-administration and to incomplete extinction. Prior operant training of the subjects was used for two reasons. First, although we wanted to ensure that animals would be opiate-naive at the start of heroin self-administration, we also wanted a rapid stabilization of heroin-reinforced responding. Without an initial period of self-administration training on a nonopiate drug, heroin self-administration can require an acquisition period of several weeks (6). Secondly, we wanted a baseline of responding for saline, as the appropriate preopiate control for naloxone-precipitated dependence measures. Without prior training on cocaine, it is likely that responding for saline would not have occurred. The relatively high number of saline infusions sustained following a week of extinction may, however, also indicate the relative inadequacy of CRF schedules for assessing the reinforcing effects of drugs.

Following prolonged treatment with opiate agonists, withdrawal reactions occur upon cessation of drug treatment or administration of an opioid antagonist (22). In the present study, the development of physical dependence on opiate was examined by observing the naloxone-precipitated withdrawal syndrome, and the intensity of the syndrome was evaluated by measuring the number of withdrawal signs and the percentage body weight loss at 4 hr after naloxone injection (1, 7, 11, 12). Although significantly more withdrawal signs and greater percentage body weight losses were observed in rats following IV self-administration of heroin at 0.1, 0.3 or 0.6 mg/kg/infusion than after saline, none of the animals exhibited diarrhea upon injection of naloxone. This is very different from previous observations in rats in which opiate dependence was induced by drinking a morphine solution (7, 11, 12). In those investigations, diarrhea was a frequently observed withdrawal sign in response to naloxone injection. It has been reported that different withdrawal signs develop at different rates and are maximal at different times (2, 8, 14, 15), and that the severity of withdrawal reactions is also determined by the route of administration of the opiate and the duration of exposure (2,9). Therefore, it is not reasonable to expect identical naloxone-precipitated withdrawal syndromes when opiate dependence is induced by different agonists given by different routes of administration. However, if the diarrhea observed in previous studies is peripheral in origin, the difference between the studies suggests that oral morphine self-administration may lead to greater occupation of receptors in the gastrointestinal tract than occurs after the intravenous route. Further studies are required before any firm conclusions can be made.

It has been shown previously that morphine self-administration can occur without development of physical dependence (21). In the present study, animals which had self-administered heroin at 0.03 mg/kg/infusion twice daily for a total of 9 sessions did not show a withdrawal syndrome after injection of 1 mg/kg naloxone. This observation provides support for the use of this concentration of heroin if the reinforcing efficacy of the drug is to be studied in the absence of physical dependence (5,6).

The influence of physical dependence on opiate self-administration has not been studied thoroughly. The present work revealed that, in response to self-administration of heroin at a unit dose of 0.03 mg/kg/infusion, opiate-dependent animals exhibited a significantly greater number of active lever presses and higher heroin intake than during their initial-dose exposure. The presence of physical dependence thus appears to lead to greater drug taking (9). This might reflect tolerance to the reinforcing effects of drugs, so that more drug is needed to attain the same desired effect (partial extinction effect). Some evidence for such tolerance has been found in studies of amphetamine self-administration in rats (14). However, it is also possible that the increased responding in our heroin-dependent rats does not represent tolerance to the original positive reinforcing effect, but rather, the addition of a new component of negative reinforcement by relief of incipient withdrawal symptoms. The higher concentrations of heroin might also have exerted some sedative effect in these opiate-dependent rats, even if not to the same degree as in nondependent and presumable nontolerant rats, and therefore, the reinforcing efficacy of the drug cannot be accurately estimated. It should also be

pointed out that opiate dependence (as reflected by a naloxone-precipitated withdrawal reaction) developed after the rats self-administered heroin at doses of 0.1 mg/kg/infusion and higher for 5 days. When animals were first exposed to heroin at 0.3 or 0.6 mg/kg/infusion, they may have been already opiate-dependent. If this were the case, the data on numbers of responses and drug intake obtained from these animals would not be appropriate for comparison with those obtained from the demonstrably opiate-dependent rats. In this study, the altered intake of heroin due to physical dependence may have been evident only at the 0.03 mg/kg unit dose.

The results appear to suggest that, overall, physical dependence has only a small effect on opiate self-administration, compared to the positive reinforcing effects of intravenous infusion. However, it must be remembered that the maximum total dose per session, at the highest unit dose, was less than 2 mg/kg. Therefore the degree of physical dependence was much less than that produced by more typical dependence-producing treatments with doses ranging up to 200 mg/kg (morphine). The present findings therefore do not exclude the possibility that negative reinforcement may play an important role in self-administration on such high-dose regimens.

It is probable that animals in this study experienced withdrawal on the weekends, and possibly also during the approximately 18-hour period between the second session of one day and the first session of the following day. Interestingly, however, when animals had access to each dose for one week, the number of responses on Mondays were not different from values on the succeeding days of the week, at any dose. Since withdrawal symptoms were produced by doses of 0.1 mg/kg/infusion and higher, the stability of responding over the week at 0.3 and 0.6 mg/kg/infusion suggests that there is little effect of weekend (or through-the-week) withdrawal on subsequent drug-taking behavior. This somewhat surprising observation suggests three possibilities. Although the duration over which withdrawal symptoms could be elicited in dependent animals at the completion of testing was greater than 3 days, it is possible that at earlier times and lower doses, withdrawal had a significantly shorter timecourse, and was complete before the next self-administration test occurred. Alternatively, withdrawal may not be severe enough to affect self-administration. Or, in the dependent animals in this study, processes other than withdrawal, for example, tolerance to the reinforcing or depressant effects of the drug, may be more important in regulating drug intake over the range of doses examined.

The current investigation also revealed that postdependent rats responded significantly more on the drug-reinforced lever and had higher heroin intake than during the initial-dose exposure when they self-administered heroin at the 0.03 mg/kg unit dose. These findings are not at variance with those reported by others (10,19). However, whether they are due to the history of physical dependence or to the history of reinforcement of drug-taking behavior is not clear. It is also possible that self-administration behavior had not been completely extinguished when heroin was reintroduced after saline substitution. Complete extinction of drug-seeking behavior may make relapse less likely.

In summary, the present study has shown that, using the technique of IV self-administration, 0.03 mg/kg/infusion is an acceptable dose for illustrating the reinforcing property of heroin in the absence of physical dependence, which develops when larger unit doses of heroin are employed. Intake of heroin at 0.03 mg/kg/infusion is found to increase significantly in opiate-dependent and postdependent rats.

ACKNOWLEDGEMENT

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