Pharmacology Biochemistry & Behavior, Vol. 36, pp. 393–399. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Acute Opioid Physical Dependence in Humans: Effect of Naloxone at 6 and 24 Hours Postmorphine

STEPHEN J. HEISHMAN,[1] MAXINE L. STITZER, GEORGE E. BIGELOW AND IRA A. LIEBSON

Behavioral Pharmacology Research Unit, Department of Psychiatry and Behavioral Sciences
The Johns Hopkins University School of Medicine, Baltimore, MD 21224

Received 15 December 1989

HEISHMAN, S. J., M. L. STITZER, G. E. BIGELOW AND I. A. LIEBSON. *Acute opioid physical dependence in humans: Effect of naloxone at 6 and 24 hours postmorphine*. PHARMACOL BIOCHEM BEHAV **36**(2) 393–399, 1990.—Previous studies in our laboratory have documented the occurrence of naloxone-precipitated opioid abstinence from 45 minutes to 6 hours after acute morphine administration in humans. This study extended the morphine-naloxone interval to 24 hours and examined the effect of repeated naloxone challenges on withdrawal responses. Six male nondependent opiate users participated in eight experimental sessions in which they received single IM injections of morphine (18 mg/70 kg) followed 6 and 24 hours later by challenge sessions with IM placebo or naloxone (10 mg/70 kg). Naloxone challenge at 6 hours postmorphine reversed morphine-induced miosis and subjective reports of opiate symptoms, drug high, good drug effects, and drug liking. At 24 hours postmorphine, naloxone had no effect on these measures, which had returned to premorphine levels. However, at 6 and 24 hours postmorphine, naloxone precipitated subjective symptoms and observer-rated signs of opioid abstinence. When naloxone challenge at 24 hours was preceded by naloxone at 6 hours postmorphine, the magnitude of abstinence symptoms and signs was attenuated. These data suggest that morphine-induced adaptational changes underlying the development of physical dependence persist beyond other measureable agonist effects, and that these changes are disrupted or reversed by repeated antagonist administration.

Acute physical dependence Opioid abstinence Antagonist-precipitated withdrawal Morphine Naloxone Human subjects

ACUTE opioid physical dependence has been defined as the abstinence syndrome precipitated by an opioid antagonist administered after either a single dose or a short-term infusion of an opioid agonist (22). This phenomenon is interesting pharmacologically because opioid abstinence has been traditionally thought to occur after cessation of chronic exposure to opioid agonists (10,14). However, research with various animal species (13, 19, 23, 30) and more recently with humans (1, 7–9, 16, 17) has demonstrated the occurrence of antagonist-precipitated abstinence after single injections of opioid agonists. These studies have suggested that adaptational changes underlying physical dependence begin to develop within minutes after acute agonist exposure. This phenomenon thus presents a model with which to investigate the pharmacological, behavioral, and temporal determinants of what may be the beginnings of opioid physical dependence.

Initial demonstrations from our laboratory of acute opioid dependence in humans showed that the intensity of the abstinence syndrome increased directly with increasing dose of morphine (1) or naloxone (7) when the interval between morphine pretreatment and naloxone challenge was 6 hours. Recently, we have begun to investigate the effect of varying the temporal interval between agonist and antagonist administration, while holding drug dose constant. For example, Heishman *et al.* (8) manipulated the interval between single doses of morphine and naloxone from 0 to 90 minutes in nondependent opiate users and found that precipitated abstinence was first observed 45 minutes after morphine administration. This is consistent with animal research demonstrating antagonist-precipitated withdrawal within 10–30 minutes after acute agonist administration (3, 12, 18, 25, 27, 31).

In contrast to the early time course of acute opioid physical dependence, the occurrence of antagonist-precipitated abstinence more than 6 hours after acute agonist administration has not been widely investigated. Three animal studies (5, 11, 28) reported antagonist-precipitated effects occurring 48 hours or more after single morphine injections; however, other studies have found that the maximal agonist-antagonist interval at which precipitated withdrawal could be observed was between 16–36 hours (2, 3, 12, 31). Clinical studies have reported naloxone-precipitated abstinence 24 hours after single injections of morphine in opiate-naive

[1]Requests for reprints should be addressed to Stephen J. Heishman, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

humans (16,17) and 1 week after dosing with a methadone-naloxone mixture in nondependent former opiate addicts (24). Thus, one purpose of this study was to compare the effect of naloxone at 6 and 24 hours after a single dose of morphine.

Recent animal studies have indicated that intermittent dosing of the benzodiazepine antagonist, Ro 15-1788, during periods of continuous benzodiazepine administration attenuated the intensity of antagonist-precipitated withdrawal (4,21). Similarly, it was shown that naloxone administered every other day during a 15-day period of daily methadone maintenance attenuated naloxone-precipitated abstinence signs in rhesus monkeys (20). One feature of two of these studies (4,20) was the administration of antagonist on two successive days after the chronic agonist dosing regimen. Both studies found that antagonist-precipitated abstinence was clearly attenuated following the repeated antagonist administration on day 2.

Studying the effects of repeated antagonist challenge may illuminate adaptational mechanisms underlying the development of opioid physical dependence. If prior challenge with a short-acting antagonist, such as naloxone, had no impact on the intensity of subsequent challenge, then one would infer that the effect of displacement of agonist from receptor sites was transient. However, if prior antagonist challenge altered the intensity of subsequent challenge, as suggested by the above animal studies, then a more lasting biological change would be suggested. Thus, a second purpose of this study was to determine whether the precipitated abstinence syndrome would be altered following two successive naloxone challenges in nondependent humans with a history of opiate use.

METHOD

Subjects

Participants were six male community volunteers in good physical health with documented histories of drug abuse, but without other significant psychiatric disorder, as assessed by the National Institute of Mental Health Diagnostic Interview Schedule (26). Subjects ranged in age from 24 to 37 years (mean = 30.5), in history of IV opiate use from 2 to 18 years (mean = 9.7), and in IV opiate use during the past month from 0 to 16 times (mean = 8.3). Two subjects had previously participated in opiate detoxification programs; one subject reported one treatment episode, the other, two. All subjects reported experiencing some degree of opiate withdrawal sickness in the past; however, none considered themselves currently dependent, nor were any seeking treatment for opiate addiction. Subjects gave written informed consent prior to the study and were paid for their participation.

General Procedures

During the 5-week study, subjects lived on an eight-bed behavioral pharmacology research ward which has been described (6). Following admission to the research ward, subjects were observed for abstinence signs for 3–4 days and provided a drug-free urine sample prior to the start of the study. No withdrawal signs were observed in any of the subjects as assessed by clinical observation and monitoring of vital signs by the nursing staff.

Before starting the protocol, subjects were informed that the purpose of the study was to gain a better understanding of physical dependence to opiate drugs and that they should expect to experience mild withdrawal sickness following some of the drug injections. Subjects were also told the general schedule of drug administration and that the drugs to be administered would include an opiate agonist, an opiate antagonist, and placebo. However, subjects and research staff were blind to the nature of individual drug injections.

Drugs

Commercially available morphine (15 mg/ml) was diluted in bacteriostatic saline to the desired concentration for injection. The constant morphine dose was 18 mg/70 kg. Naloxone hydrochloride (DuPont, Wilmington, DE) was weighed as the salt and dissolved in bacteriostatic saline to a concentration of 10 mg/ml, which was then diluted in saline to desired concentrations. The constant naloxone dose was 10 mg/70 kg. Placebo injections consisted of bacteriostatic saline. Doses of morphine and naloxone were adjusted for body weight, as indicated, and all injections were administered IM under double blind conditions in a constant volume of 1.5 ml in the right deltoid muscle.

Experimental Procedures

Subjects participated in ten experimental sessions; each spanned two days. Sessions were conducted on Monday–Tuesday and Thursday–Friday of each week. On the first session day, morphine was administered at 8:00 or 10:30 a.m. At 2:00 or 4:30 p.m. (6 hours postmorphine), subjects were challenged with naloxone or placebo. The next morning at 8:00 or 10:30 a.m. (24 hours postmorphine), subjects received a second injection of naloxone or placebo. Thirty minutes before each challenge injection, subjects were seated in a quiet room and connected to physiological recording equipment. While measurements stabilized, a baseline pupil photograph was taken, subjective forms completed, and signs of withdrawal observed. Baseline physiological measures were then recorded for 10 minutes. The measurement battery of pupil photographs, subjective reports, and observer-rated withdrawal signs was repeated at 5, 15, 30, 45, and 60 minutes after the challenge injection.

During the first experimental session, subjects received morphine placebo; 6 hours later, they received naloxone and 24 hours later, placebo. The purpose of this session was to verify further the absence of opioid physical dependence by testing for naloxone-precipitated abstinence signs and symptoms; none were observed in any subject. During the second session, subjects received morphine followed 6 hours later by naloxone and 24 hours later by placebo. The purpose of this session was to determine each subject's sensitivity to the antagonist challenge. Six subjects reported symptoms and exhibited signs of naloxone-precipitated abstinence following 18 mg/70 kg morphine pretreatment. One subject was insensitive to the antagonist challenge and was dismissed from the study following session 2. Subjects then participated in eight sessions in which naloxone or placebo challenges occurred 6 and 24 hours postmorphine. Subjects were exposed twice in a randomized block design to four treatment conditions, summarized in Table 1. Data from the two exposures to each treatment condition were averaged for each subject.

Physiological Measures

Systolic and diastolic blood pressure, mean arterial pressure, heart rate, respiratory rate, and skin temperature were recorded continuously throughout challenge sessions as previously described (7). Pupil photographs were taken in ambient room lighting using a Polaroid camera with 3× magnification.

Subjective Measures

During challenge sessions, subjects completed opioid symptom, withdrawal symptom, and drug effect questionnaires. The

TABLE 1

SUMMARY OF EXPERIMENTAL CONDITIONS

Treatment Condition	Postmorphine Challenge Sessions 6 Hours	24 Hours
1	Placebo	Placebo
2	Naloxone	Placebo
3	Placebo	Naloxone
4	Naloxone	Naloxone

opioid and withdrawal symptom forms each contained 15 items describing typical opioid or withdrawal effects (8). The drug effect questionnaire assessed six items: drug high, any drug effect, good drug effect, bad drug effect, drug liking, and withdrawal sickness. Subjects rated the extent to which they currently experienced each symptom or effect on a 10-point scale from 0 (not at all) to 9 (most strongly). A composite score was obtained for the withdrawal and opioid symptom questionnaires by summing the 15 items. Drug effect questionnaire items were scored separately.

Observer Measures

Lacrimation (tearing eyes), rhinorrhea (runny nose), perspiration, piloerection (gooseflesh), yawning, and restlessness were observed as previously described (7) by a research technician who was blind to drug. These abstinence signs were rated on the same 10-point scale used for subjective report measures. A composite observer rating score was obtained by summing the individual item scores.

Data Analysis

Measures analyzed included pupillary diameter, respiratory rate, heart rate, skin temperature, systolic and diastolic blood pressure, mean arterial pressure, composite score on the opioid and withdrawal symptom questionnaires, individual withdrawal symptom and drug effect questionnaire items, and individual and composite scores of observer-rated withdrawal signs. Data consisted of the prechallenge baseline measurement and the area under the time course curve (AUC) of the 60-minute postchallenge session. Because baseline data differed at 6 and 24 hours postmorphine for some measures, AUC data for all measures were expressed as difference from prechallenge baseline values. Difference scores were analyzed by two-way repeated measures analysis of variance (ANOVA) with treatment condition (placebo-placebo, naloxone-placebo, placebo-naloxone, naloxone-naloxone) and time postmorphine (6 and 24 hours) as the factors. Huynh-Feldt adjustments of repeated measure degrees of freedom were used to correct for violation of the sphericity assumption. On measures which revealed a significant main effect or interaction, post hoc comparisons between and within specific treatment conditions were conducted using the Tukey method. Questions addressed in post hoc testing were: 1) Were naloxone effects different from placebo at 6 and 24 hours postmorphine?, 2) Was the intensity of naloxone effects different at 6 and 24 hours?, and 3) Was the 24-hour naloxone challenge differentially affected by the 6-hour naloxone or placebo challenge? For all statistical tests, effects were considered significant if $p<0.05$.

RESULTS

Reversal of Agonist Effects

Figure 1 shows pupillary diameter and composite responses on the 15-item opioid symptom questionnaire as a function of the naloxone challenge at 6 hours postmorphine (condition 2, see Table 1), the 24-hour naloxone challenge that was preceded by placebo at 6 hours (condition 3), and respective placebo challenges from condition 1. These representative measures illustrate the reversal of residual agonist effects by naloxone at 6 hours postmorphine. Immediately before the naloxone injection, pupils were constricted to 3.1 mm from a premorphine diameter of 5.8 mm. Post hoc analysis indicated that naloxone significantly increased pupillary diameter (1.8 mm from prenaloxone baseline) compared to placebo, fully reversing the morphine-induced miosis. By 24 hours postmorphine, pupillary diameter had returned to premorphine levels (5.5 mm), and thus naloxone had no effect. Similar changes were observed for respiratory rate, although the increase by naloxone at 6 hours postmorphine (1.7 breaths/minute from prenaloxone baseline) just missed statistical significance.

Figure 1 also indicates that naloxone significantly reversed residual opioid symptoms that subjects were reporting at 6 hours postmorphine before the naloxone challenge. At 24 hours post-

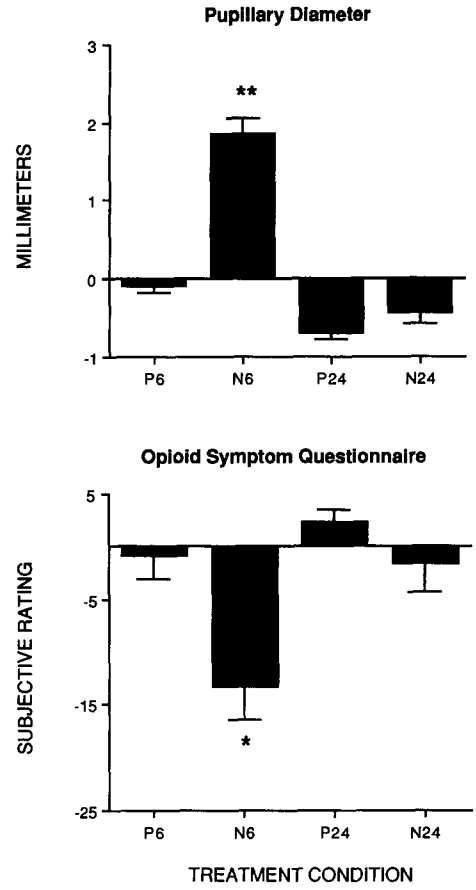

FIG. 1. Opioid reversal effect of naloxone (10 mg/70 kg) or placebo on pupillary diameter and opioid symptom questionnaire in subjects pretreated with a single dose of morphine (18 mg/70 kg) as a function of the following treatment conditions at 6 or 24 hours postmorphine: P6 [placebo at 6 hours, condition 1 (see Table 1)], N6 (naloxone at 6 hours, condition 2), P24 (placebo at 24 hours, condition 1), N24 (naloxone at 24 hours, condition 3). Data are mean difference ± 1 S.E.M. from prechallenge baseline of area under the time course curve of 60-minute postchallenge session for six subjects. Significant differences from respective placebo conditions are indicated by $*p<0.05$ and $**p<0.01$.

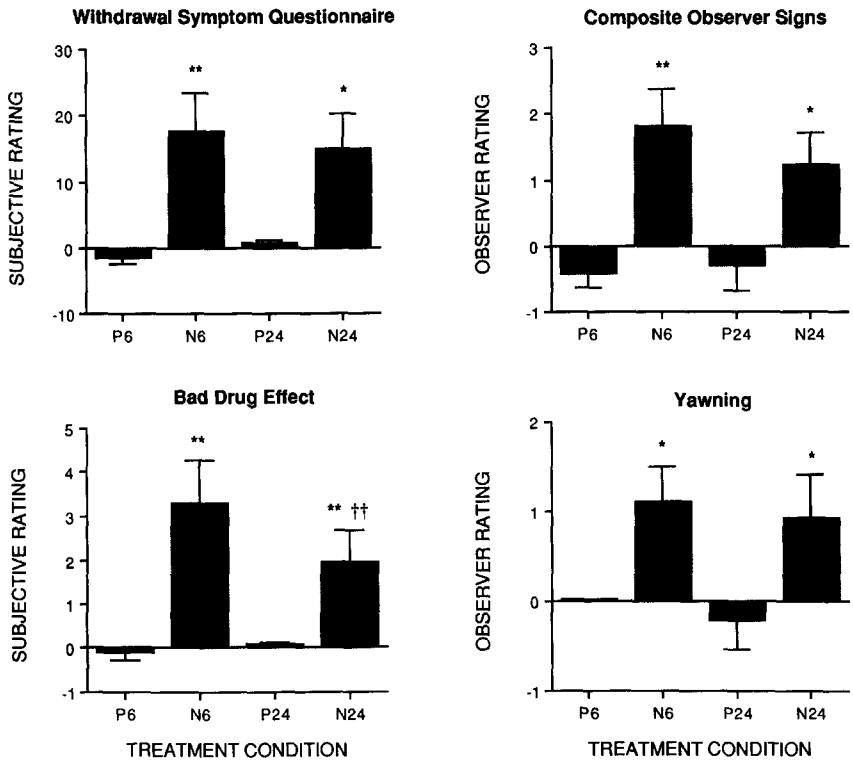

FIG. 2. Antagonist-precipitated abstinence effect of naloxone (10 mg/70 kg) or placebo on subjective symptoms (left column) and observer-rated signs (right column) in subjects pretreated with a single dose of morphine (18 mg/70 kg) as a function of 6- or 24-hour postmorphine conditions (see Fig. 1 legend for explanation of treatment conditions). Data are mean difference ±1 S.E.M. from prechallenge baseline of area under the time course curve of 60-minute postchallenge session for six subjects. Significant differences from respective placebo conditions are indicated by $*p<0.05$ and $**p<0.01$. Significant difference from 6-hour naloxone challenge is indicated by $\dagger\dagger p<0.01$.

morphine, subjective agonist effects were no longer reported, and naloxone had no effect. A similar pattern of naloxone-induced reversal of opioid effects at 6 hours and lack of naloxone effect at 24 hours postmorphine was observed for the drug high, good drug effect, and drug liking items from the drug effect questionnaire.

Antagonist-Precipitated Abstinence: Single Naloxone Challenge

Figure 2 shows two subjective and two observer measures as a function of the naloxone session at 6 hours postmorphine (condition 2), the 24-hour naloxone challenge that was preceded by placebo at 6 hours (condition 3), and respective placebo challenges from condition 1 (same treatment conditions as Fig. 1). Post hoc analysis indicated that subjective responses on the 15-item withdrawal symptom questionnaire and the bad drug effect item from the drug effect questionnaire were significantly increased by naloxone at 6 and 24 hours postmorphine compared to placebo. Similarly, naloxone significantly increased ratings on the withdrawal sickness item from the drug effect questionnaire at 6 and 24 hours postmorphine. The magnitude of responses on the withdrawal symptom questionnaire did not differ between the 6- and 24-hour naloxone challenges; however, ratings of bad drug effect and withdrawal sickness were significantly greater at 6 than 24 hours postmorphine. Naloxone also significantly increased composite observer ratings of opioid abstinence signs at 6 and 24 hours postmorphine relative to placebo; yawning was the only individual sign that was significantly elevated (Fig. 2). For both observer measures, the magnitude of the abstinence rating was not significantly different between the 6- and 24-hour naloxone sessions.

The following individual items on the withdrawal symptom questionnaire were comparably elevated at 6 and 24 hours postmorphine after naloxone challenge: backache, upset stomach, yawning, watery eyes, hot or cold feelings, and irritable. Ratings of muscle cramps, abdominal cramps, and clammy and damp skin were slightly more intense at 6 compared to 24 hours, whereas reports of restlessness were of greater magnitude at 24 than 6 hours postmorphine. Painful joints, runny nose, sneezing, chills or gooseflesh, and bothered by noises were minimally reported after naloxone challenge.

Significant changes relative to placebo in two physiological measures also reflected naloxone-precipitated abstinence. Heart rate increased 2.3 beats/minute and skin temperature decreased 1.8 degrees F. from prenaloxone baseline after naloxone at 6 hours postmorphine. No effect on heart rate or skin temperature was observed after the 24-hour naloxone challenge. The three blood pressure measures were not significantly affected by naloxone at either 6 or 24 hours postmorphine.

Antagonist-Precipitated Abstinence: Repeated Naloxone Challenge

Figure 3 shows the same subjective and observer measures from Fig. 2 as a function of the 24-hour naloxone challenge that was preceded by placebo at 6 hours postmorphine (condition 3) and the 24-hour naloxone challenge that was preceded by naloxone at 6 hours (condition 4). Following the repeated naloxone chal-

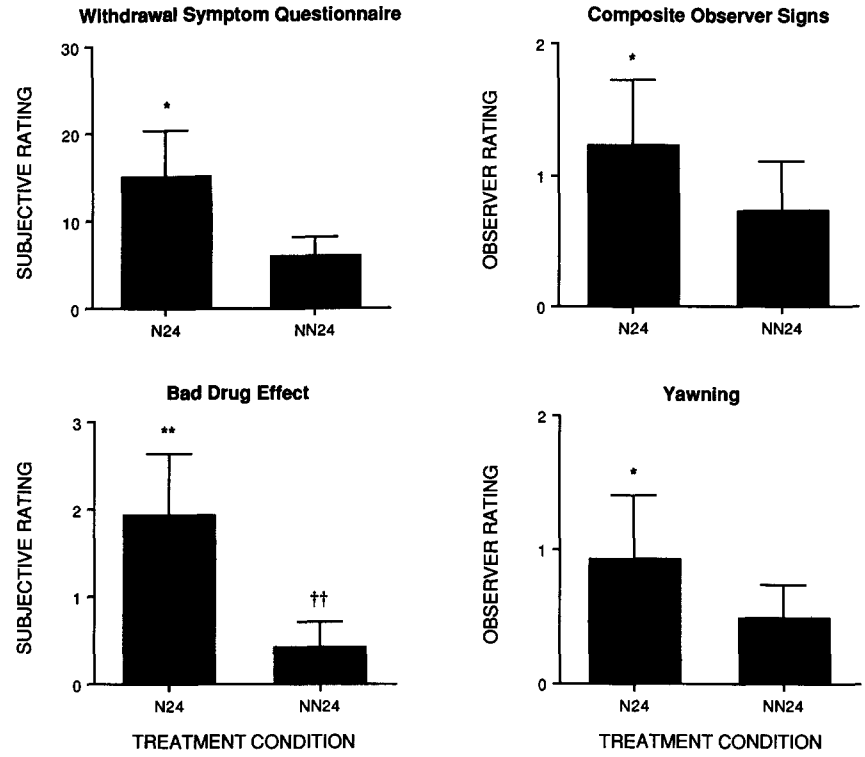

FIG. 3. Antagonist-precipitated abstinence effect of naloxone (10 mg/70 kg) on subjective symptoms (left column) and observer-rated signs (right column) in subjects pretreated with a single dose of morphine (18 mg/70 kg) as a function of the following treatment conditions at 24 hours postmorphine: N24 (naloxone at 24 hours preceded by placebo at 6 hours, condition 3) and NN24 (naloxone at 24 hours preceded by naloxone at 6 hours, condition 4). Data are mean difference ± 1 S.E.M. from prechallenge baseline of area under the time course curve of 60-minute postchallenge session for six subjects. Significant differences from respective placebo conditions (shown in Fig. 2) are indicated by $*p<0.05$ and $**p<0.01$. Significant difference from N24 condition is indicated by $††p<0.01$.

lenge, the magnitude of precipitated abstinence symptoms and signs was clearly attenuated. Post hoc analysis indicated that subjective responses on the withdrawal symptom questionnaire and bad drug effect item were not significantly different from placebo. Identical effects were seen with the withdrawal sickness item. Additionally, ratings of bad drug effect and withdrawal sickness after the repeated challenge were significantly less than after the single naloxone challenge at 24 hours postmorphine. Figure 3 also shows that the composite observer rating and observer-rated yawning were attenuated in the repeated naloxone condition. The attenuation seen with the observer ratings was not as large as that of the subjective measures, but, again, the observer ratings in the repeated naloxone condition were not significantly different from placebo.

DISCUSSION

This study documented the occurrence of naloxone-precipitated opioid abstinence at 6 and 24 hours after a single dose of morphine in nondependent opiate users. This is consistent with recent reports of acute opioid physical dependence testing a 6-hour morphine-naloxone interval in humans (1, 7–9). To our knowledge, this is the first report of precipitated abstinence at 24 hours postmorphine in opiate users. Jones (16,17) reported naloxone-precipitated withdrawal 24 hours after single morphine injections in opiate-naive subjects. This suggests that the time course of acute physical dependence may be similar in the two populations. However, a systematic comparison of agonist and antagonist effects between opiate-experienced and opiate-naive persons would be needed to characterize potential population differences in sensitivity to the pharmacological effects of opioids, including acute physical dependence.

The precipitated opioid abstinence syndrome was generally similar at 6 and 24 hours postmorphine, with some indication of reduced intensity at 24 hours. Subjective ratings of two individual items, bad drug effect and withdrawal sickness, were significantly greater at 6 compared to 24 hours postmorphine, and naloxone-induced changes in heart rate and skin temperature observed at 6 hours were not evident after the 24-hour challenge. However, composite subjective ratings on the withdrawal symptom questionnaire and composite observer ratings of abstinence signs did not significantly differ in intensity at the two times (Fig. 2), although there was a trend toward decreased intensity at 24 hours. The qualitative nature of the precipitated abstinence syndrome also did not differ at 6 and 24 hours postmorphine, in that similar abstinence symptoms and signs were reported or observed. At both postmorphine times, subjects reported general dysphoria and sickness and specific abstinence symptoms, including yawning, hot or cold feelings, upset stomach, irritability, abdominal cramps, watery eyes, and backache. Yawning was the only abstinence sign consistently observed after naloxone at either 6 or 24 hours postmorphine. This constellation of abstinence symptoms and

signs is consistent with previous reports of antagonist-precipitated abstinence in humans after acute (1, 7–9, 16, 17) or chronic (15,29) exposure to opioids and with reports of spontaneous abstinence following chronic opioid administration (10).

The results of this study, together with previous work from our laboratory (8), now indicate that naloxone-precipitated abstinence can occur as early as 45 minutes and at least as long as 24 hours after a single dose of morphine in nondependent opiate users. At 24 hours postmorphine, agonist effects, such as miosis, respiratory depression, and subjective opioid symptoms, were no longer measureable. This is consistent with morphine's relatively short half-life in plasma of 2.5–3 hours and the fact that 90% of a single dose is excreted within 24 hours (14). That precipitated abstinence was observed at 24 hours suggests that morphine induced some physiological or biochemical adaptational change persisting beyond its observable effects, which may represent the beginnings of opioid physical dependence. Some animal studies (5, 11, 28) have reported that antagonist-precipitated abstinence occurred at postmorphine intervals longer than 24 hours when larger doses of either agonist or antagonist were used. Thus, it is possible that precipitated abstinence could be observed in humans at longer postagonist intervals if larger doses of agonist and/or antagonist were used. Additionally, naloxone-precipitated abstinence has been observed in humans 1 week after a single 40 mg dose of methadone (24). The half-life of methadone in humans is 24–36 hours (14), which implies that the time course of antagonist-precipitated abstinence can also be extended with a longer acting agonist.

The second major finding of this study was that the naloxone-precipitated abstinence syndrome at 24 hours postmorphine was attenuated when preceded by naloxone challenge at 6 hours. This reduction was reflected in subjective symptoms and observer-rated signs of opioid abstinence (Fig. 3). Similar attenuation of precipitated abstinence after the second of two successive antagonist administrations has been reported following chronic dosing with diazepam (4) and methadone (20) in rhesus monkeys. These studies also reported that intermittent antagonist dosing during chronic agonist administration attenuated the development of physical dependence, as measured by reduced precipitated abstinence following the chronic dosing regimen. These animal data and those of the present study are consistent with the hypothesis that antagonist administration resets or reverses receptor mechanisms for the development of physical dependence, such that repeated antagonist challenge in the absence of continued agonist administration, as in this study, results in the attenuation of precipitated abstinence. However, as stated above, it is possible that with a longer acting agonist, larger doses of agonist or antagonist, or a shorter interval between the first and second antagonist challenge, one might observe repeated antagonist-precipitated abstinence after acute agonist treatment.

In summary, this study has documented the occurrence of naloxone-precipitated opioid abstinence in nondependent opiate users 24 hours after single morphine injections, when residual agonist effects were no longer measureable. Further, this study showed that the abstinence syndrome was attenuated following two successive naloxone challenges. The morphine-induced adaptational changes underlying acute precipitated abstinence are not currently understood; however, knowledge of such mechanisms may be an important element in understanding the development of opioid physical dependence.

ACKNOWLEDGEMENTS

This research was supported by USPHS Research Grant DA-04011 and Research Training Grant DA-07209 from the National Institute on Drug Abuse. The authors thank Beverly Banks for assistance in conducting the study and Linda Felch for consultation and assistance with data analysis. A preliminary report of this research was presented at the 51st Annual Scientific meeting of the Committee on Problems of Drug Dependence, Inc.; Keystone, CO; June, 1989.

REFERENCES

1. Bickel, W. K.; Stitzer, M. L.; Liebson, I. A.; Bigelow, G. E. Acute physical dependence in man: Effects of naloxone after brief morphine exposure. J. Pharmacol. Exp. Ther. 244:126–132; 1988.
2. Cheney, D. L.; Goldstein, A. Tolerance to opioid narcotics: Time course and reversibility of physical dependence in mice. Nature 232:477–478; 1971.
3. Eisenberg, R. M. Further studies on the acute dependence produced by morphine in opiate naive rats. Life Sci. 31:1531–1540; 1982.
4. Gallager, D. W.; Heninger, K.; Heninger, G. Periodic benzodiazepine antagonist administration prevents benzodiazepine withdrawal symptoms in primates. Eur. J. Pharmacol. 132:31–38; 1986.
5. Gellert, V. F.; Sparber, S. B. A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. J. Pharmacol. Exp. Ther. 201:44–54; 1977.
6. Griffiths, R. R.; Bigelow, G. E.; Liebson, I. A.; Kakiszak, J. E. Drug preference in humans: Double-blind choice comparison of pentobarbital, diazepam and placebo. J. Pharmacol. Exp. Ther. 215:649–661; 1980.
7. Heishman, S. J.; Stitzer, M. L.; Bigelow, G. E.; Liebson, I. A. Acute opioid physical dependence in postaddict humans: Naloxone dose effects after brief morphine exposure. J. Pharmacol. Exp. Ther. 248:127–134; 1989.
8. Heishman, S. J.; Stitzer, M. L.; Bigelow, G. E.; Liebson, I. A. Acute opioid physical dependence in humans: Effect of varying the morphine-naloxone interval. I. J. Pharmacol. Exp. Ther. 250:485–491; 1989.
9. Higgins, S.; Preston, K.; Cone, E.; Henningfield, J.; Jaffe, J. Behavioral, physiological and hormonal effects of a naloxone challenge following acute morphine pretreatment in humans. In: Harris, L. S., ed. Problems of drug dependence 1987. Washington, DC: U.S. Government Printing Office; 1988:202–208.
10. Himmelsbach, C. K. The morphine abstinence syndrome, its nature and treatment. Ann. Intern. Med. 15:829–839; 1941.
11. Huidobro, F. Some relations between tolerance and physical dependence to morphine in mice. Eur. J. Pharmacol. 15:79–84; 1971.
12. Jacob, J. J.; Barthelemy, C. D.; Tremblay, E. C.; Colombel, M. L. Potential usefulness of single-dose acute physical dependence on and tolerance to morphine for the evaluation of narcotic antagonists. In: Braude, M. C.; Harris, L. S.; May, E. L.; Smith, J. P.; Villarreal, J. E., eds. Advances in biochemical psychopharmacology. vol. 8. Narcotic antagonists. New York: Raven Press; 1974:299–318.
13. Jacob, J. J.; Michaud, G. M. Acute physical dependence in the waking dog after a single low dose of morphine. Psychol. Med. 4:270–273; 1974.
14. Jaffe, J. H.; Martin, W. R. Opioid analgesics and antagonists. In: Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F., eds. The pharmacological basis of therapeutics. New York: Macmillan; 1985: 491–531.
15. Jasinski, D. R. Assessment of the abuse potentiality of morphinelike drugs (methods used in man). In: Martin, W. R., ed. Handbook of experimental pharmacology. vol. 45. Drug addiction I. Berlin: Springer-Verlag; 1977:197–258.
16. Jones, R. T. Dependence in non-addict humans after a single dose of morphine. In: Way, E. L., ed. Endogenous and exogenous opiate agonists and antagonists. New York: Pergamon Press; 1980:557–560.
17. Jones, R. T. Caffeine enhances morphine dependence in humans. In: Takagi, H.; Simon, E., eds. Advances in endogenous and exogenous opioids. New York: Elsevier; 1981:472–474.
18. Kosersky, D. S.; Harris, R. A.; Harris, L. S. Naloxone-precipitated jumping activity in mice following the acute administration of morphine. Eur. J. Pharmacol. 26:122–124; 1974.

19. Krystal, J. H.; Redmond, D. E. A preliminary description of acute physical dependence on morphine in the vervet monkey. Pharmacol. Biochem. Behav. 18:289–291; 1983.
20. Krystal, J. H.; Walker, M. W.; Heninger, G. R. Intermittent naloxone attenuates the development of physical dependence on methadone in rhesus monkeys. Eur. J. Pharmacol. 160:331–338; 1989.
21. Lamb, R. J.; Griffiths, R. R. Effects of repeated Ro 15-1788 administration in benzodiazepine-dependent baboons. Eur. J. Pharmacol. 110:257–261; 1985.
22. Martin, W. R.; Eades, C. G. A comparison between acute and chronic physical dependence in the chronic spinal dog. J. Pharmacol. Exp. Ther. 146:385–394; 1964.
23. Meyer, D. R.; Sparber, S. B. Evidence of possible opiate dependence during the behavioral depressant action of a single dose of morphine. Life Sci. 21:1087–1094; 1977.
24. Nutt, J. G.; Jasinski, D. R. Methadone-naloxone mixtures for use in methadone maintenance programs. I. An evaluation in man of their pharmacological feasibility. II. Demonstration of acute physical dependence. Clin. Pharmacol. Ther. 15:156–166; 1974.
25. Ritzmann, R. F. Opiate dependence following acute injections of morphine and naloxone: The assessment of various withdrawal signs. Pharmacol. Biochem. Behav. 14:575–577; 1981.
26. Robins, L. N.; Helzer, J. E.; Croughan, J.; Ratcliff, K. S. The NIMH diagnostic interview schedule: Its history, characteristics and validity. Arch. Gen. Psychiatry 38:381–389; 1981.
27. Smits, S. E. Quantitation of physical dependence in mice by naloxone-precipitated jumping after a single dose of morphine. Res. Commun. Chem. Pathol. Pharmacol. 10:651–661; 1975.
28. Tremblay, E.; Colombel, M.; Jacob, J. Precipitation et prevention de l'abstinence chez le Rat et la Souris en etat de dependance aigue. Comparaison de la naloxone, de la naltrexone et de la diprenorphine. Psychopharmacology (Berlin) 49:41–48; 1976.
29. Wikler, A.; Fraser, H. F.; Isbell, H. N-allylnormorphine: Effects of single doses and precipitation of acute "abstinence syndromes" during addiction to morphine, methadone or heroin in man (post-addicts). J. Pharmacol. Exp. Ther. 109:8–20; 1953.
30. Wiley, J. N.; Downs, D. A. Naloxone-precipitated jumping in mice pretreated with acute injections of opioids. Life Sci. 25:797–801; 1979.
31. Yano, I.; Nishimo, H.; Murano, T. Antagonism by naloxone of tolerance and dependence in mice given a single dose of morphine. Jpn. J. Pharmacol. 29:357–366; 1979.