Pharmacology Biochemistry & Behavior, Vol. 13, pp. 115–119. Printed in the U.S.A.

Narcotic Abstinence in Dependent Rats: EEG and Behavioral Correlates[1]

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Received 31 January 1980

YOUNG, G. A., G. F. STEINFELS AND N. KHAZAN. *Narcotic abstinence in dependent rats: EEG and behavioral correlates.* PHARMAC. BIOCHEM. BEHAV. 13(1) 115–119, 1980.—The purpose of the study was to assess and compare EEG and behavioral correlates of abstinence from morphine, methadone, LAAM, NLAAM, and DNLAAM in dependent rats. Rats were initially trained to lever press for self-injections on a FR-20 schedule of reinforcement. Following substitution of saline for each of the narcotic agonists, REM sleep was significantly suppressed during morphine, methadone, and NLAAM abstinence, but not during LAAM and DNLAAM abstinence. Significant increases in lever pressing for saline during abstinence from morphine, methadone, and NLAAM emerged earlier than with LAAM and DNLAAM. Significant increases in head-shake behavior occurred during morphine, methadone, NLAAM, and DNLAAM abstinence, but not during LAAM abstinence. These results demonstrated further pharmacodynamic differences between the five narcotics studied. Our findings suggested that in dependent rats abstinence from LAAM was the least severe when compared with abstinence from any of the other four narcotics studied.

Morphine Methadone LAAM NLAAM DNLAAM Self-administration Abstinence
REM sleep Lever pressing Head shakes

SEVERAL electroencephalographic (EEG) and behavioral correlates of morphine self-maintained dependence and subsequent abstinence have been previously characterized in the rat. Self-administered morphine in dependent rats has been shown to produce a brief episode of cortical EEG slow-bursts associated with a stuporous behavior [16,17]. This phase was followed by behavioral arousal, and then by slow-wave sleep (SWS) and rapid eye movement (REM) sleep episodes that predominated until immediately before lever pressing and the next morphine self-injection. The interinjection intervals during morphine self-administration were of two to three hours' duration.

These same variables have been studied and compared during dependence on l-alpha-acetylmethadol (LAAM), methadone, and morphine [20]. The pattern of distributions of arousal, SWS, and REM sleep during the interinjection intervals of the rats dependent on these three narcotic agonists was in general similar. Injection intervals were 2.5±0.1 hr (mean±s.e.m.) during morphine self-administration and 1.4±0.1 and 8.8 ± 0.8 hr for methadone and LAAM, respectively. In a similar study during self-maintained dependence on either morphine or methadone by the rat, head shakes appeared and increased in frequency before lever pressing for injections [1]. In contrast, there were fewer head shakes during LAAM dependence, and these head shakes were evenly distributed over the entire duration of the inter-injection interval.

More recently, we studied dependent rats self-administering LAAM and each of its two active N-demethylated metabolites, nor-LAAM (NLAAM), and dinor-LAAM (DNLAAM) [25]. We found that self-injections of NLAAM resulted in immediate suppression of SWS and REM sleep. A similar suppression of SWS and REM sleep occurred only occasionally after LAAM injections; however, this effect was rarely seen after DNLAAM injections. Although mean durations of the inter-injection intervals were approximately 5 hr (1 mg/kg dose) for all three narcotics, injections were more evenly spaced during NLAAM and DNLAAM self-administration than during LAAM self-administration. The frequency of lever pressing and head-shake behavior increased in the latter portion of the interinjection interval during NLAAM and DNLAAM self-administration. In the case of LAAM self-administration, the increase in lever pressing prior to a self-injection was not accompanied by a similar increase in head-shake behavior.

The physiological and behavioral correlates of the abstinent state produced by withdrawal from morphine in dependent rats have also been extensively studied. Abstinence produced by substitution of saline for morphine led to increasing rates of lever pressing [17,23] that peaked in about 7 hr after the last morphine injection and then declined [17]. Moreover, REM sleep episodes were severely suppressed for at least 24 hr following morphine withdrawal and then returned to normal in a few days [17]. Morphine abstinence

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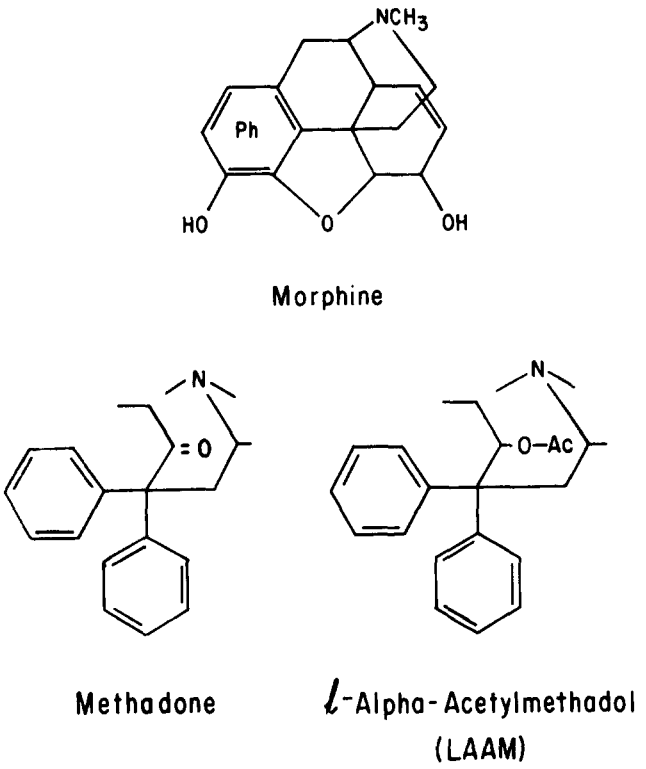

FIG. 1. Chemical structures of morphine, methadone, and l-alpha-acetylmethadol (LAAM). NLAAM and DNLAAM are the N-demethylated metabolites of LAAM.

has also been correlated with increased irritability or aggression [4, 6, 9], body weight losses [3,19], increases in "wet-dog shakes" [3,19], decreases in EEG integrated voltage output [13], and REM sleep suppression followed by a rebound [14]; similar REM sleep effects occurred during methadone abstinence [15].

In a comparative study REM sleep was severely suppressed during the first 24 hr of abstinence from morphine and methadone, but only moderately suppressed with LAAM [27]. In addition, increases in lever pressing during abstinence from morphine and methadone in dependent rats occurred earlier and were more frequent and prolonged than for LAAM. The incidence of head shakes peaked earlier and was higher for morphine and methadone during abstinence than for LAAM. In a later study, however, when abstinence was induced by intravenous (IV) naloxone injections, REM sleep occurrences were suppressed to a similar degree and for similar durations during precipitated abstinence in morphine and LAAM-dependent rats [26].

The purpose of the present study was to extend our previous comparative evaluations of LAAM, NLAAM, and DNLAAM in dependent rats [25] by assessing the behavioral and EEG correlates that emerge during abstinence from these narcotics. These evaluations were also contrasted with those during abstinence from morphine and methadone.

METHOD

Twenty-one female Sprague-Dawley rats (250–300 g) were used. For drug injections, a silicone rubber cannula was implanted under ketamine anesthesia (100–150 mg/kg, intraperitoneal) into the right external jugular vein [21,22]. For bipolar EEG recordings, stainless steel screws were implanted over the frontal and parietal cortices. For electromyographic (EMG) recordings, stainless steel wires were inserted into the temporalis muscles. Electrodes were soldered to a miniature Continental connector which was attached to the skull with dental acrylate.

The rats were maintained in individual cages that contained a response lever, and permitted drug administration and the continuous recording of EEG and EMG activities. To allow free movement of the rat, each cage was equipped with a swivel cable connector having concentric pools of mercury as noise-free contacts for EEG and EMG recordings, and a feed-through cannula for drug administration. Lighting conditions consisted of a timer-regulated light period from 6 a.m. to 10 p.m. Lever presses and drug injections were recorded on a Esterline-Angus event recorder as well as on the event marker channels of the Grass polygraph, which recorded the EEG and EMG activities continuously. The total number of injections per 24 hr was recorded on mechanical counters [12].

Morphine sulfate, methadone hydrochloride, LAAM hydrochloride, NLAAM hydrochloride, and DNLAAM hydrochloride (Fig. 1) were dissolved in physiological saline and delivered by electronically controlled Harvard syringe drivers. Rats were made tolerant and physically dependent by a series of hourly automatic IV morphine injections [2]. Each rat was then trained to lever press on a fixed-ratio schedule of reinforcement to receive 10 mg/kg IV morphine injections (3 sec injections of 0.05 ml). A fixed ratio (FR) of one lever press per injection was initially required and gradually increased to FR-20. When the daily number of morphine self-injections had stabilized at 8–12 injections/day, the rats were divided among the following five groups: (1) four rats continued to self-administer morphine in the same manner; (2) in five other rats methadone (2 mg/kg per injection) was substituted for morphine on the FR-20 schedule; (3) in four other rats LAAM (1 mg/kg per injection) was substituted for morphine on the FR-20 schedule; (4) in four other rats NLAAM (1 mg/kg per injection) was substituted for morphine on the FR-20 schedule; (5) in the four remaining rats DNLAAM (1 mg/kg per injection) was substituted for morphine on the FR-20 schedule. These relative doses of morphine, methadone, LAAM, NLAAM, and DNLAAM usually result in a single or double self-injection with a cessation of further lever pressing with no signs of drug toxicity [20,25]. When the daily number of injections had stabilized in the five groups of rats (in about one week), physiological saline was substituted for each of the five narcotics and offered on the same FR-20 schedule. EEG and EMG recordings were made continuously for at least one week of abstinence. REM sleep, lever pressing, and head-shake correlates of abstinence were studied in these five groups of rats.

Occurrences of head shakes were determined by observing the polygraphic correlate of this behavior. Head shakes produced artifacts in EEG tracings that consisted of wide sweeps of the ink pen [3,27]. Periodic observations confirmed that these head-shake artifacts were usually a component of "wet-dog shakes" [19].

RESULTS

REM sleep and behavioral data during the last 24 hr of self-administration of morphine, methadone, LAAM, NLAAM, and DNLAAM, and during the first 48 hr of absti-

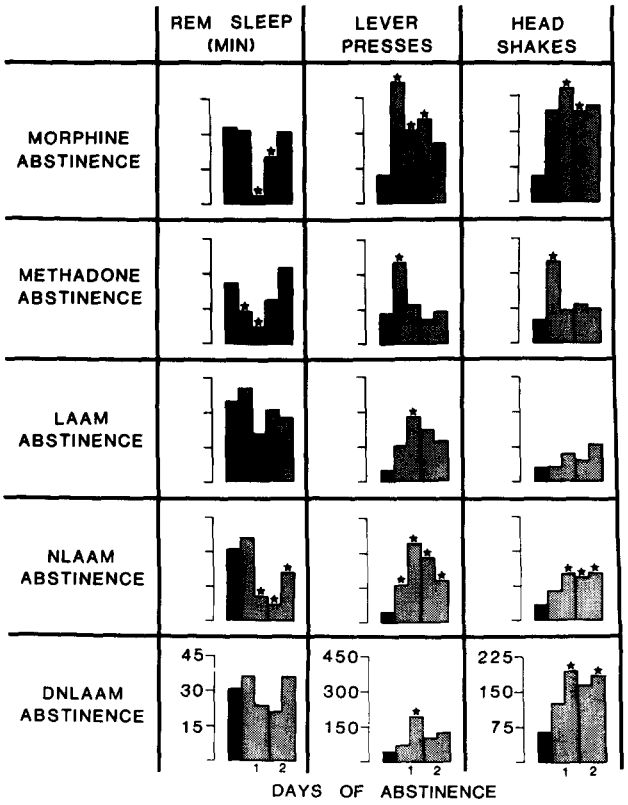

FIG. 2. REM sleep, lever pressing, and head shake correlates are shown during abstinence from morphine, methadone, LAAM, NLAAM, and DNLAAM in dependent rats. Mean values per 12 hr during the last 24 hr of self-administration are indicated by the darkened bars in the left of each histogram. The shaded portion of each histogram represents mean values per consecutive 12 hr block during abstinence. Stars indicate significant differences between self-administration values and abstinence values; $p<0.01$.

nence are shown in Figure 2. Each set of data was subjected to a subjects×treatments analysis of variance. For example, mean REM sleep times were compared in 12 hr blocks during morphine self-administration and abstinence. Fifteen separate analyses of variance were performed. When a significant F value was obtained, Newman-Keuls tests on differences between all possible pairs of means were performed [24]. The rejection region for all statistical tests was set at the 1% level. Significant differences of greatest interest were those between self-administration values and any abstinence values; these are indicated by the stars in Fig. 2.

Upon withdrawal from morphine, REM sleep (left column of Fig. 2) was significantly suppressed, compared to self-injection values, from the 12th to 36th hr after the last morphine self-injection. In the cases of methadone and NLAAM, REM sleep was also significantly suppressed, compared to self-administration values, during the first 24 hr and from the 12th to 24th hr, respectively, after the last narcotic self-injection. In contrast, with LAAM and DNLAAM abstinence, REM sleep was not significantly suppressed.

Lever pressing was significantly increased, compared to self-administration values, during the first 36 hr of morphine abstinence (middle column of Fig. 2). In the cases of methadone and NLAAM abstinence, lever pressing was also significantly increased during the first 12 and 48 hr, respectively. In the cases of LAAM and DNLAAM abstinence, significant increases in lever pressing were relatively delayed in onset and were manifested from the 12th to 24th hr.

Head shakes were significantly increased, compared to self-administration values, after 12 hr of abstinence from morphine, NLAAM, and DNLAAM (right column of Fig. 2). In the case of methadone abstinence, head shakes were significantly increased during the first 12 hr. In contrast, during LAAM abstinence head shakes did not significantly increase in number.

DISCUSSION

The present findings demonstrated that withdrawal from methadone in methadone-dependent rats was associated with a relatively smaller increase in lever pressing and less head-shake behavior when compared with morphine withdrawal. However, the onset of REM sleep suppression was sooner during withdrawal from methadone than from morphine. Although clinical reports have also indicated that withdrawal from methadone is less intense than withdrawal from morphine, clinical reports have indicated, in contrast to our experimental findings, that withdrawal from methadone develops more slowly and is more prolonged than withdrawal from morphine. These differences between our experimental results and the clinical reports may be related to the relatively shorter duration of action of methadone given intravenously to rats compared to the relatively longer duration of action of methadone when given orally to humans.

The results of our study also demonstrated that the abstinence syndrome after withdrawal from LAAM in dependent rats was less severe than that from either morphine or methadone. In contrast with morphine or methadone, withdrawal from LAAM was not associated with any significant REM sleep suppression or any significant increase in head shakes. This difference in the degree of intensity of the abstinence syndrome between LAAM and morphine or methadone may have been the result of a lower level of physical dependence produced by LAAM compared with that produced by morphine and methadone. However, since it was previously found that naloxone-induced abstinence in morphine and LAAM-dependent rats resulted in a similar degree of REM sleep suppression [26], the relatively mild abstinence syndrome found in the present study and that was reported earlier [27] is most likely related to the relatively long plasma half-lives of the two pharmacologically active N-demethylated LAAM metabolites, NLAAM, and DNLAAM [8, 11, 18]. For example, in the female rat following the acute administration of 5 mg/kg of LAAM, plasma levels of LAAM peaked at 30 sec, and the early phase half-life for LAAM was approximately 8 hr [7]. Furthermore, peak plasma NLAAM levels occurred at 12 hr following LAAM dosing, and peak plasma DNLAAM levels were reached 24 hr after LAAM injection. Detectable amounts of NLAAM and DNLAAM persisted for up to 96 hr.

Abstinence from NLAAM resulted in a degree of REM sleep suppression similar to that with morphine and methadone. These three narcotics have also been found to exert an immediate REM sleep suppressant effect after self-injection during self-administration [20,25]. In contrast, abstinence from LAAM and DNLAAM did not produce any significant REM sleep suppression. Furthermore, neither LAAM nor DNLAAM disrupted diurnal variations in REM sleep distributions during self-administration [25].

Significant increases in lever pressing during abstinence from LAAM and DNLAAM were delayed compared to those with morphine, methadone, and NLAAM. As noted

above, LAAM and DNLAAM abstinence was not associated with any significant suppression of REM sleep. Therefore, since the degree of REM sleep suppression during narcotic abstinence may reflect relative severity of abstinence, the delayed appearance of increased lever pressing during LAAM and DNLAAM abstinence may also reflect a difference in severity of abstinence.

Finally, during morphine, methadone, NLAAM, and DNLAAM abstinence, head shakes significantly increased in number compared to self-administration values. In contrast, during LAAM abstinence head shakes did not significantly increase in number. In previous studies we found that during morphine, methadone, NLAAM, and DNLAAM self-administration head shakes increased in frequency in the latter portion of interinjection intervals, while during LAAM self-administration head shakes did not increase in frequency in the latter portion of interinjection intervals [1,25]. It was suggested that if plasma and brain levels of a respective narcotic became low enough before the rat lever-pressed for an injection, increases in number of head shakes in the latter portion of interinjection intervals may have reflected early signs of withdrawal. With LAAM, the metabolism of LAAM to NLAAM and eventually to DNLAAM may have sustained a relatively more stable behavioral state during self-maintained dependence than that associated with the other narcotics studied. This could have accounted for the absence of any abrupt increase in frequency of head-shake behavior during LAAM self-administration. Therefore, one might predict that the emergence of head shakes during withdrawal from LAAM would be more gradual and less severe than during withdrawal from morphine, methadone, NLAAM, and DNLAAM. These predictions were supported by our data.

In summary, the results of this study demonstrated further pharmaco-dynamic differences between morphine, methadone, LAAM, NLAAM, and DNLAAM. Our findings suggest that in dependent rats abstinence from LAAM was the least severe when compared with abstinence from any one of the other four narcotics studied.

ACKNOWLEDGEMENT

Thanks are due to Mr. Michael Gentry for technical assistance and to Ms. Barbara Baker for the preparation and the typing of the manuscript.

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