Pharmacology Biochemistry & Behavior, Vol 10, pp 585–589 Printed in the U S A

Spontaneous vs. Naloxone-Induced Abstinence in Dependent Rats Self-Administering L-Alpha-Acetylmethadol (LAAM) or Morphine[1]

GERALD A. YOUNG,[2] GEORGE F. STEINFELS AND NAIM KHAZAN

Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore MD 21201

(Received 25 August 1978)

YOUNG, G. A., G F STEINFELS AND N KHAZAN *Spontaneous vs naloxone-induced abstinence in dependent rats self-administering l-alpha-acetylmethadol (LAAM) or morphine* PHARMAC. BIOCHEM. BEHAV 10(4) 585–589, 1979.—Rats maintained dependence by the self-administration of LAAM or morphine Following the substitution of saline for LAAM, REM sleep was not disrupted, and the frequency of lever pressing for saline self-injections peaked at about 24 hr In contrast, following the substitution of saline for morphine, REM sleep was suppressed for 24 hr while the frequency of lever pressing for saline self-injections peaked within 8 hr When abstinence was induced by hourly *iv* naloxone injections, REM sleep occurrences were suppressed to a similar degree and for similar durations during naloxone-induced abstinence from both morphine and LAAM These results suggest that the level of physical dependence maintained during self-administration of LAAM and morphine was similar The relatively mild abstinence syndrome that was seen during saline substitution in LAAM-dependent rats was most likely related to the long plasma half-lives of the pharmacologically active N-demethylated metabolites of LAAM

LAAM Self-Administration Abstinence REM sleep Lever pressing

PREVIOUS reports have contained descriptions of behavioral and electroencephalographic (EEG) correlates during self-administration of and abstinence from *l*-alpha-acetylmethadol (LAAM), morphine and methadone in the dependent rat [1, 9, 11, 14] Abstinence from LAAM was associated with less lever pressing during saline substitution, less disruption of rapid eye movement (REM) sleep, and fewer head shakes than abstinence from morphine and methadone. Irritability scores increased for morphine and methadone during the first day of abstinence but did not show any increase until the third day for LAAM 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. An alternative explanation might be that the milder abstinence syndrome associated with withdrawal from LAAM was related to the relatively long plasma half-lives of the two active N-demethylated LAAM metabolites, nor-LAAM and dinor-LAAM [3, 4, 10]. In this case, naloxone treatment should precipitate a more severe abstinence syndrome in a LAAM-dependent rat than was seen in the earlier study [14]. On the other hand, if LAAM self-maintained dependence was associated with a lower level of physical dependence, then naloxone-induced abstinence in LAAM-dependent rats should still be of lower intensity than that in morphine and methadone-dependent rats

Previous studies showed that the degree and time course of REM sleep suppression during morphine abstinence coincided with occurrences of hyperirritability, piloerection, ptosis, diarrhea, and decreases in mean EEG voltage output [6,9]. Thus, disruptions in REM sleep provide an index of the severity and duration of a narcotic abstinence syndrome. In an attempt to delineate possible differences in the levels of physical dependence with morphine and LAAM, naloxone-induced abstinence was precipitated in LAAM and in morphine-dependent rats and the degree of REM sleep disruption was measured and compared

METHOD

Seventeen female Sprague-Dawley rats (250–300 g) were used. For drug injections, a chronic silicone rubber cannula was implanted under ketamine anesthesia (100–150 mg/kg, *ip*) into the right external jugular vein [12,13] For bipolar EEG recordings, stainless steel screws were implanted over the ipsilateral frontal and parietal cortices [5]. For electromyographic (EMG) recordings, stainless steel wires were inserted into the temporalis muscles Electrodes were soldered to a miniature Continental connector which was at-

[1]Supported by NIDA grant DA-01050

[2]Reprint requests to Gerald A Young, Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, 636 West Lombard Street, Baltimore MD 21201

TABLE 1
MEAN REM SLEEP TIMES (MIN/24 HOURS)

	Spontaneous Abstinence		*Naloxone-Induced Abstinence*		*Naloxone Control*
	Morphine (10 mg/kg/inj)	LAAM (1 mg/kg/inj)	Morphine (10 mg/kg/inj)	LAAM (1 mg/kg/inj)	Naloxone (0 5 mg/kg/hr)
Day 1	21 88	70 00	1 75	1 17	75 17
Day 2	48 10	74 00	30 25	14 17	69 33
Day 3	63 94	75 63	50 17	43 58	74 33
Day 4	66 56	79 13	70 58	62 25	74 00

tached to the skull with dental acrylate and Eastman 910 adhesive

Throughout the study each rat was maintained in an individual cage that was equipped with a response lever, a swivel cable connector for EEG and EMG recordings and a feed-through cannula for drug administration [5,9] Lighting conditions consisted of a timer-regulated period of darkness from 10 p.m to 6 a.m EEG and EMG activities were recorded continuously on a Grass Model 7D polygraph. The EEG was filtered to pass frequencies between 1 and 35 Hz Lever presses and drug injections were recorded on an Esterline-Angus event recorder as well as on the event marker channels of the Grass polygraph

Morphine sulfate and LAAM hydrochloride were dissolved in physiological saline and delivered by electronically-controlled Harvard syringe drivers Fourteen rats were made tolerant to and physically dependent on morphine by a series of hourly automatic intravenous injections During the first day rats received 1.25 mg/kg/hr of morphine The dose was increased to 2 5, 5 0, 10 0 and 20 0 mg/kg/hr on successive days Each of these rats was then trained to lever press on a fixed ratio (FR) schedule of reinforcement to receive morphine (10 mg/kg/injection, 0 05 ml over 3 sec). An FR of one lever press was initially required per injection which was gradually raised to FR-20. When the daily number of morphine self-injections had stabilized at 8–12 injections per day, in seven of the rats LAAM (1 mg/kg/injection) was substituted for morphine. After stabilized responding for both LAAM and morphine had been established, saline was substituted for both LAAM and morphine in four rats in each group In the other six rats, automatic hourly *iv* injections of naloxone (0 5 mg/injection) were delivered for seven days, LAAM or morphine self-injections were no longer available In three additional drug-naive rats, similar naloxone injections were delivered for seven days

The main parameter studied was the distribution of REM sleep episodes during LAAM and morphine self-administration and during saline substitution or naloxone-induced abstinence Lever pressing pattern of behavior was also studied during self-administration and during saline substitution The rejection level for all statistical analyses was set at the 5% level Behavioral states of sleep, REM sleep and wakefulness were identified by the corresponding changes in EEG and EMG recordings [6,7]

RESULTS

REM sleep data obtained during self-administration of morphine and LAAM and during spontaneous abstinence are shown in the left side of Table 1 The rats averaged 60 25 ± 6 03 (mean ± s.e m.) min of REM sleep during the last day of morphine self-administration and 70 81 ± 3.36 min of REM sleep during the last day of LAAM self-administration REM sleep data obtained during spontaneous abstinence from morphine and LAAM were compared with an analysis of variance The main treatment variables were drug group (morphine vs LAAM) and day of abstinence (Day 1 through Day 4). There were significant effects of both drug group and day of withdrawal on REM sleep time. The rats engaged in more REM sleep during spontaneous abstinence from LAAM than from morphine, $F(1,6)=36\ 30$, and engaged in more REM sleep during the last two days of abstinence studied than during the first one or two days, $F(3,18)=26\ 23$ The drug group x day of withdrawal interaction factor was also significant, $F(3,18)=12\ 84$. An examination of the data in the left side of Table 1 suggests that this interaction occurred because REM sleep times increased at a higher rate over the four days of abstinence from morphine than during abstinence from LAAM

Self-injection data obtained during spontaneous abstinence from morphine and LAAM are shown in Fig 1 The rats took an average of 12.25 ± 0 73 morphine injections during the last day of self-administration and an average of 3 75 ± 0.87 LAAM injections during the last day of self-administration Saline self-injection data obtained during spontaneous abstinence from morphine and LAAM were compared with an analysis of variance. The main treatment variables were drug group (morphine vs LAAM) and successive 6-hr periods for two days after abstinence (1 through 8) There was a significant effect of time after initiation of spontaneous abstinence on the number of saline self-injections The rats took more saline injections during the first one or two 6-hr periods during spontaneous abstinence than during later 6-hr periods, $F(7,42)=7.82$. The drug group × 6-hr period interaction factor was also significant, $F(7,42)=14\ 40$ An examination of the data in Fig 1 suggests that this interaction occurred because the number of saline self-injections was the highest during the first 12 hr of spontaneous withdrawal from morphine, while the number of saline self-injections was the highest between the 18th and 30th hr after spontaneous abstinence from LAAM The drug group variable was not significant

The pattern of morphine self-injections and REM sleep distribution with one of the morphine-dependent rats is shown in the top of Fig 2 In this particular case single injections of morphine were taken on the average of every 2 to 3 hr Furthermore, morphine self-injections typically suppressed occurrences of REM sleep for 30 min or more, after which they reappeared and persisted until just before each subsequent self-injection Similar observations have been

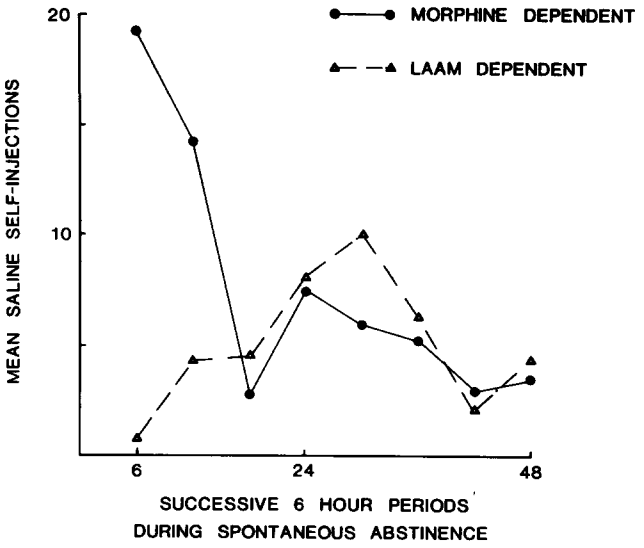

FIG 1 Mean number of saline self-injections are shown as a function of successive 6-hr periods during spontaneous abstinence from morphine and LAAM in dependent rats

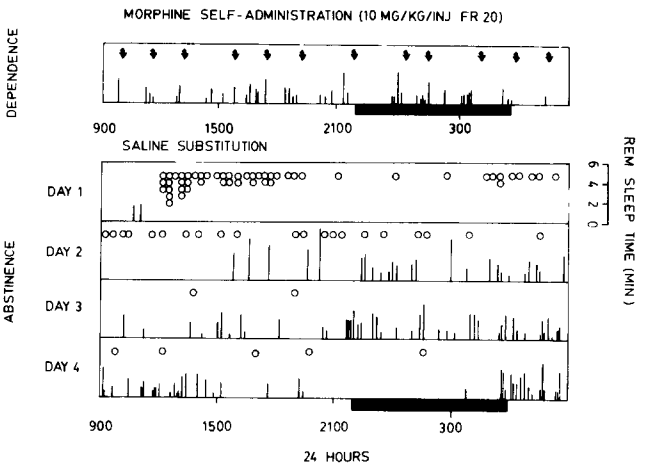

FIG 2 REM sleep distribution for the last day of self-maintained dependence on morphine and the first four days after substitution of saline for morphine Morphine self-injections are indicated by the arrows Saline self-injections are indicated by the open circles The timer-regulated period of darkness is indicated on the 24 Hours scale

previously reported [8, 9, 11]. During the substitution of saline for morphine, REM sleep was completely suppressed for the first 24 hr Although REM sleep occurrences reappeared during the next three days, daily amounts of REM sleep were still below normal levels. The frequency of saline self-injections peaked within 6–8 hr after the last morphine injection.

The pattern of LAAM self-injections and REM sleep distribution with one of the LAAM-dependent rats is shown in the top of Fig 3 In this example, single injections of LAAM were taken about every 8 hr REM sleep occurrences and

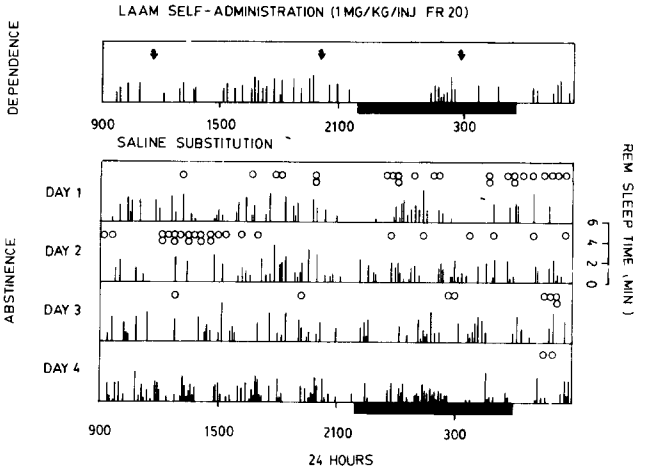

FIG 3 REM sleep distribution for the last day of self-maintained dependence on LAAM and the first four days after the substitution of saline for LAAM LAAM self-injections are indicated by the arrows Saline self-injections are indicated by the open circles The timer-regulated period of darkness is indicated on the 24 Hours scale

LAAM self-injections were not related to one another in any apparent manner. Substitution of saline for LAAM did not result in any suppression of REM sleep. There was a gradual increase in the daily amount of REM sleep over the four days following saline substitution. Self-injections of saline gradually increased in frequency during the first 24 hr of abstinence and reached a peak in frequency at about 24 hr.

REM sleep data obtained during self-administration of morphine and LAAM and during naloxone-induced abstinence are shown in the middle of Table 1 The rats averaged 57 00 ± 5.88 min of REM sleep during the last day of morphine self-administration and 63.58 ± 6.80 min of REM sleep during the last day of LAAM self-administration. The REM sleep data obtained during naloxone-induced abstinence were compared in an analysis of variance. The main treatment variables were drug group (morphine vs LAAM) and day of abstinence (Day 1 through Day 4). There was a significant effect of day of abstinence on REM sleep time. The rats engaged in more REM sleep in the later days of abstinence than in the earlier days, $F(3,12)=8$ 27. The drug group variable and the interaction factor were not significant. Data obtained during naloxone administration in control rats is shown in the right side of Table 1. The rats averaged 75 25–6 96 min of REM sleep per day while receiving automatic *iv* injections of saline There was no apparent effect of naloxone treatment on REM sleep in these control rats

The amount and distribution of lever pressing during naloxone-induced abstinence from morphine and LAAM did not differ. For example, the rats averaged 263 ± 99 lever presses during the first day of naloxone-induced abstinence from morphine and 219 ± 35 lever presses during the first day of naloxone-induced abstinence from LAAM.

The pattern of morphine self-injections and REM sleep distribution with one of the morphine-dependent rats is shown in the top of Fig 4. During naloxone-induced abstinence from morphine REM sleep was continuously suppres-

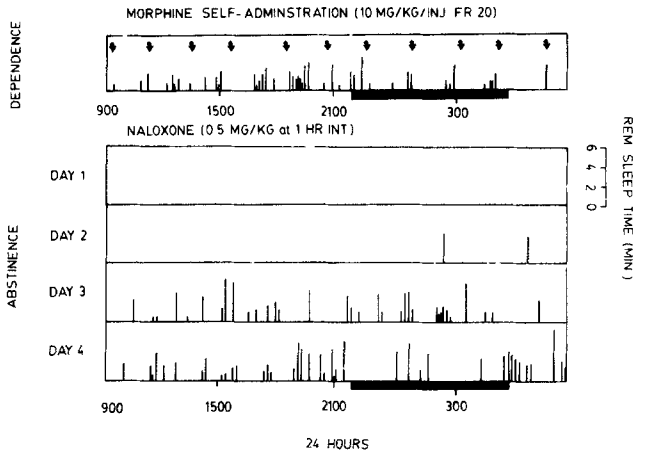

FIG 4 REM sleep distribution for the last day of self-maintained dependence on morphine and the first four days of naloxone-induced abstinence. Morphine self-injections are indicated by the arrows During abstinence naloxone (0 5 mg/kg, *iv*) was automatically administered at one-hour intervals The timer-regulated period of darkness is indicated on the 24 Hours scale

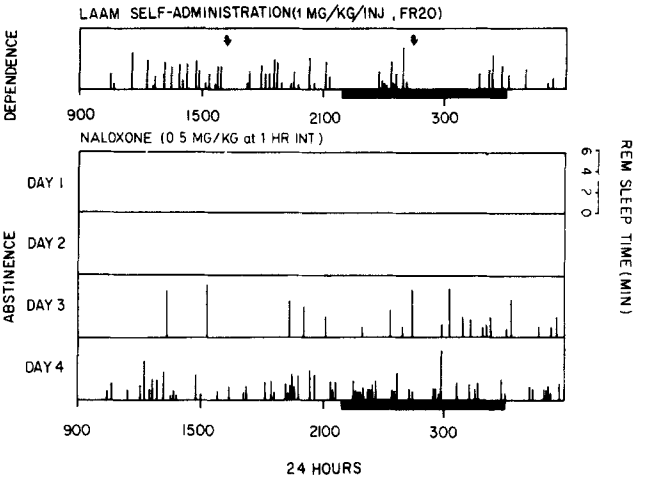

FIG 5 REM sleep distribution for the last day of self-maintained dependence on LAAM and the first four days of naloxone-induced abstinence LAAM self-injections are indicated by the arrows During abstinence naloxone (0.5 mg/kg, *iv*) was automatically administered at one-hour intervals The timer-regulated period of darkness is indicated on the 24 Hours scale

sed during the first day, and only two episodes emerged during the second day

The pattern of LAAM self-injections and REM sleep distribution with one of the LAAM-dependent rats is shown in the top of Fig. 5 Naloxone-induced abstinence in this rat produced a complete suppression of REM sleep for two days The reappearance of REM sleep during the third and fourth days of abstinence from LAAM resembled that which occurred with morphine

DISCUSSION

The study demonstrated that naloxone-induced abstinence in morphine and LAAM-dependent rats resulted in a similar degree of REM sleep suppression. This finding suggests that morphine and LAAM offer similar levels of physical dependence. Thus, the relatively mild abstinence syndrome found in the present study and that was reported earlier [14] after the substitution of saline for LAAM, compared to that for morphine and methadone, is most likely related to the relatively long plasma half-lives of the two pharmacologically active N-demethylated LAAM metabolites, nor-LAAM and dinor-LAAM [3, 4, 10]

The pattern of lever pressing during spontaneous abstinence also differed between morphine- and LAAM-dependent rats. Self-injections of saline peaked within the first 12 hr during spontaneous abstinence from morphine and between the 18th and 30th hr after abstinence from LAAM These results are similar to those previously reported [14] Patterns of lever pressing did not differ during naloxone-induced abstinence from morphine and LAAM. It also appeared that the amount of lever pressing, in general, was greater during spontaneous abstinence than during naloxone-induced abstinence Observations of the rats indicated that they were experiencing more physical distress during naloxone-induced abstinence than during spontaneous abstinence That is, more wet-dog shaking, abdominal stretching, diarrhea, etc., were evident during naloxone-induced abstinence. The greater degree of physical distress during naloxone-induced abstinence may have contributed to partial suppression of lever pressing behavior. In a similar vein, it has been reported that more aggression occurred during spontaneous abstinence than during naloxone-induced abstinence in morphine-dependent rats [2]

REFERENCES

1 Chiba, S , G A Young, J E Moreton and N Khazan Head-shake distributions during self-maintained dependence on morphine, methadone and *l*-alpha-acetylmethadol (LAAM) in the rat *Psychopharmacology* **54:** 105–107, 1977

2 Gianutsos, G , M D Hynes, R B Drawbaugh and H Lal Paradoxical absence of aggression during naloxone-precipitated morphine withdrawal. *Psychopharmacology* **43:** 43–46, 1975

3 Henderson, G L , J A Weinberg, W A Hargreaves, D H M Lau, J Tyler and B Baker Accumulation of *l*-α-acetylmethadol (LAAM) and active metabolites in plasma following chronic administration *J Anal Toxicol* **1:** 1–5, 1977.

4 Kaiko, R. F and C E Inturrisi Disposition of acetylmethadol in relation to pharmacologic action *Clin Pharmac Ther* **18:** 96–103, 1975

5 Khazan, N The implication and significance of EEG and sleep-awake activity in the study of experimental drug dependence on morphine In *Methods in Narcotics Research (Modern Pharmacology-Toxicology, Vol 5)*, edited by S Ehrenpreis and A Neidle. New York Marcel Dekker, Inc , 1975, pp. 173–215

6 Khazan, N. and B Colasanti. Decline in the mean integrated electroencephalogram voltage during morphine abstinence in the rat *J Pharmac exp Ther* **177:** 491–499, 1971

7 Khazan, N and B Colasanti Protracted rebound in rapid eye movement sleep time and electroencephalogram voltage output in morphine-dependent rats upon withdrawal *J Pharmac exp Ther* **183:** 23–30, 1972

8 Khazan, N and J R. Weeks. The electroencephalogram and the electromyogram of self-maintained morphine addicted rats in relation to injections *Pharmacologist* **10:** 189, 1968
9 Khazan, N , J R Weeks and L A Schroeder. Electroencephalographic, electromyographic and behavioral correlates during a cycle of self-maintained morphine addiction in the rat *J Pharmac exp Ther* **155:** 521–531, 1967
10 Lau, D H M and G. L. Henderson Plasma levels of *l*-α-acetylmethadol (LAAM) and metabolites following acute and chronic oral administration in man *Proc West Pharmac Soc* **18:** 270–274, 1975
11 Moreton, J E., T Roehrs and N. Khazan. Drug self-administration and sleep-awake activity in rats dependent on morphine, methadone, or l-alpha-acetylmethadol *Psychopharmacology* **47:** 237–241, 1976
12 Weeks, J R Experimental morphine addiction Methods for automatic intravenous injections in unrestrained rats. *Science* **138:** 143–144, 1962
13. Weeks, J R. Long-term intravenous infusion In *Methods in Psychobiology, Vol 2,* edited by R D Myers New York Academic Press, 1972, pp 155–168.
14 Young, G A , J E Moreton, L T Meltzer and N. Khazan *l*-alpha-acetylmethadol (LAAM), methadone, and morphine abstinence in dependent rats EEG and behavioral correlates *Drug Alc Dependence* **2:** 141–148, 1977