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Opioid Self-Administration in Rats: Pharmacodynamics and Pharmacokinetics[1]

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YOUNG, G. A. AND N. KHAZAN. *Opioid self-administration in rats: Pharmacodynamics and pharmacokinetics.* PHARMACOL BIOCHEM BEHAV 27(2) 373–377, 1987.—This article provides comparative data obtained during opioid self-administration in rats, using our EEG-EMG rat model of addiction. This model allows continuous recording of EEG and EMG activities and programming of intravenous drug injections. Comparative data on opioid self-administration patterns are presented. These studies on the association between EEG and behavioral correlates of opioid self-administration have contributed to the delineation of similarities and differences in pharmacodynamic and pharmacokinetic characteristics of opioids.

Opioid self-administration EEG Pharmacodynamics Pharmacokinetics

FROM the beginning of recorded history man has used drugs for non-medical purposes. However, it was not until the 1960s that drug self-administration techniques were established for laboratory animals [7, 21, 29]. Mainly rhesus monkeys, dogs and rats have been utilized for such studies. It has been shown that a high correlation exists between drugs that are self-administered by man and by laboratory animals [11,12].

During the last two decades electroencephalographic (EEG) and behavioral techniques have been utilized by Khazan and associates to investigate the accompanying electrophysiological and behavioral changes during the self-administration of many opioids in rats. An early study demonstrated that morphine-dependent rats prepared with cortical EEG and nuchal muscle electrodes would self-administer 10 mg/kg doses of morphine via chronic intravenous cannulae at regular intervals of two to three hr [17]. Characteristic changes in EEG and in the sleep-awake cycle during morphine self-administration in the rat were also defined [15].

The following overview will focus on the pharmacodynamic and pharmacokinetic properties of opioids during self-administration, using the same EEG-EMG rat model of addiction developed earlier [17].

METHODOLOGY

A detailed review of the procedures used in the preparation of female Sprague-Dawley rats with chronic EEG and electromyographic (EMG) electrodes and permanent indwelling intravenous cannulae was previously published (see Fig. 1) [15]. This same review also described significant changes in EEG and behavior associated with the development of morphine tolerance, and the states of morphine dependence and abstinence. A detailed account of the processing of EEG samples with power spectral analyses has also been reported [16,37].

RESULTS

Comparative Opioid Self-Administration

Self-injection patterns of morphine, methadone and LAAM. Morphine-dependent rats, having continuous 24 hr access to morphine (10 mg/kg/inj), approached the lever for self-injection on the average of every 2–3 hr [19]. These rats usually self-administered single, but occasionally two or three closely spaced morphine injections (see Fig. 2). When methadone (2 mg/kg/inj) was substituted for morphine, there was a significant ($p<0.001$) shift towards shorter interinjection intervals, i.e., 1–2 hr. However, the onset of methadone-induced effects on EEG and behavior was more rapid and more intense than with morphine, presumably due to its relatively higher lipid solubility. Attempts to increase the duration of methadone interinjection intervals by increasing its dose to 4 mg/kg often resulted in toxic manifestations, which included intense stupor and muscle rigidity, and severe respiratory depression. LAAM self-administration (1 mg/kg/inj) was characterized by frequent multiple injections and relatively long interinjection intervals (6–8 hr). These data reflected relative differences in the potencies and durations of action of morphine, methadone and LAAM when self-administered intravenously by the opioid-dependent rat.

It has been clinically established that the N-demethylated metabolites of LAAM, NLAAM and DNLAAM, are active opioids with relatively long plasma half-lives [13,14]. Thus, the relatively long duration of action of LAAM is most likely due to its conversion to long-acting active metabolites. Accordingly, we found that injection patterns during NLAAM

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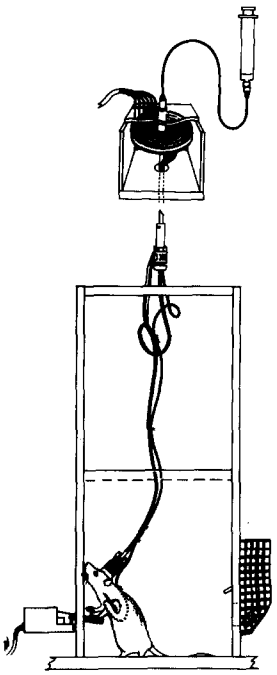

FIG. 1. The experimental cage of a rat chronically implanted with cortical and myographic electrodes and with a silicone rubber cannula. (From [15], by courtesy of Marcel Dekker, Inc.)

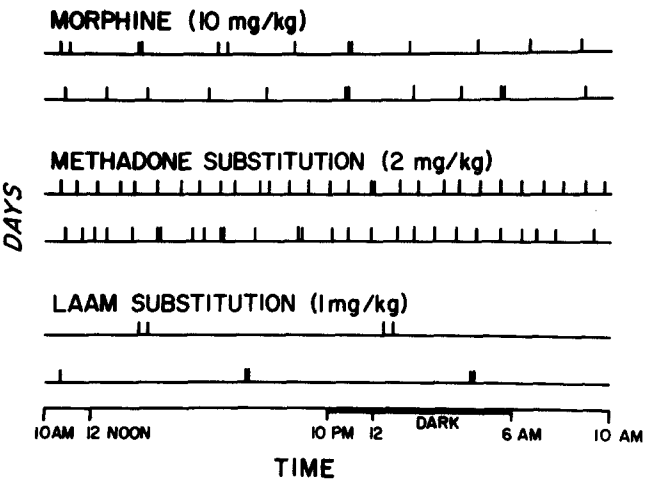

FIG. 2. Injection patterns of a representative rat during self-administration of morphine, methadone, or LAAM. Two consecutive days are shown for each drug. Small vertical bars represent injections on FR 20 schedule of reinforcement. (From [19], by courtesy of Springer-Verlag.)

and especially DNLAAM self-administration (1, 2 and 4 mg/kg/inj) in the rat demonstrated more evenly spaced interinjection intervals with fewer occurrences of multiple injections when compared to LAAM self-administration [33].

In the case of *kappa* opioid agonists, one issue that has been controversial is their possible abuse potential. It has been reported that ketocyclazocine (KC) and ethylketocyclazocine (EKC) are not self-administered by monkeys [30]. In contrast, primary self-administration of EKC by the rat has been observed [8]. We studied possible substitution of either KC or EKC (2.5 mg/kg/inj) for morphine in rats maintaining their own dependence by self-administration [31]. When either KC or EKC was substituted for morphine, rats self-administered single injections at relatively evenly spaced intervals (1–2 hr) over a 24-hr period. These self-injection patterns remained stable for up to at least 15 consecutive days.

EEG correlates of opioid self-administration. The comparative pharmacodynamic effects of morphine, methadone and LAAM on overt behavior and on the distribution of sleep-awake behavior within the interinjection interval during self-administration are of interest and have been compared in previous studies [17,19]. In naive or non-tolerant rats, morphine initially produced wakefulness, characterized by behavioral stupor or catalepsy. This depressed behavioral phase was later followed by behavioral stimulation, characterized by increased locomotor activity and stereotypy. Concomitant with the initial depressed phase, the EEG tracing showed the occurrence of high-voltage EEG slow-wave bursts. During the subsequent phase of stimulation, the EEG revealed desynchronized low-voltage tracings. On the other hand, in dependent rats self-administering morphine, each self-injection produced brief episodes of behavioral stupor and EEG slow-wave bursts followed by an extended period of wakefulness. Sleep and REM sleep then reappeared and predominated before the next self-injection. There were no apparent signs of withdrawal prior to any self-injections. The sleep-awake distribution during the interinjection intervals for methadone and LAAM was qualitatively similar to that for morphine. However, after LAAM self-injections, brief periods of sleep and REM sleep often persisted before the appearance of the behavioral stupor and EEG slow-wave bursts [19]. The delayed emergence of LAAM effects was probably correlated with a gradual accumulation of the pharmacologically active LAAM metabolites, NLAAM and DNLAAM, whereas morphine and methadone metabolites were not active.

EEG power spectral correlates of opioid self-administration. The first REM sleep episode which appeared after each self-injection of morphine in a dependent rat exhibited the faster predominant EEG frequency as measured by spectral analyses. During successive REM sleep episodes in an interinjection interval, as time progressed toward another morphine self-injection, a linear decline of mean EEG frequencies occurred [38]. The slowing of the EEG frequency persisted when saline was substituted for morphine. When morphine injections were again taken, the higher frequency of the REM sleep EEG was reinstated. Significant linear declines in mean EEG frequencies during successive REM sleep episodes were also observed during self-administration of methadone, LAAM, NLAAM, and DNLAAM (see Fig. 3). However, with morphine and methadone these declines were significantly steeper than with LAAM, NLAAM, and DNLAAM [23]. In general, the linear declines in mean EEG frequencies probably reflect declining opioid brain levels.

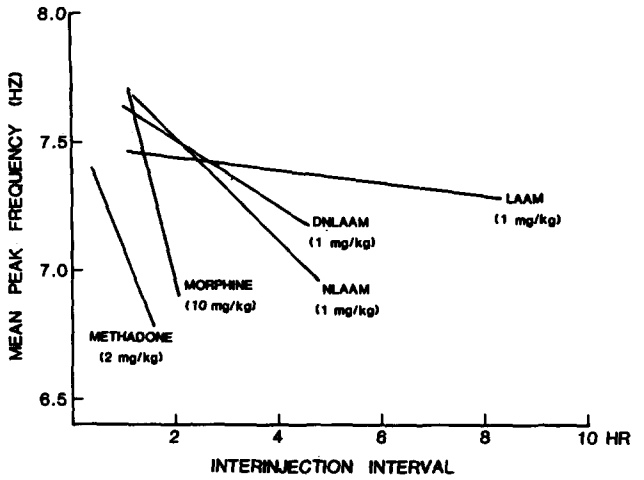

FIG. 3. Mean EEG peak frequencies (Hz) of successive REM sleep episodes during opioid interinjection intervals are shown as best-fit regression lines. The length of each line relative to the x-axis indicates the average duration between self-injections of the respective drug (interinjection interval). (From [23], by courtesy of Pergamon Press Ltd.)

Transition from morphine or methadone self-administration to LAAM or NLAAM self-administration. Reports of clinical studies related to transition from methadone to LAAM maintenance have indicated that during the early days of LAAM treatment, many human addicts were especially nervous, tense, edgy, irritable, or had difficulties in sleeping [4, 13, 14, 26]. These transient symptoms of distress may have been due to the relatively low plasma levels of the N-demethylated active metabolites of LAAM found early in treatment. However, after repeated administration of LAAM three times a week, NLAAM and DNLAAM plasma levels increased four- to ten-fold [4, 13, 14]. These increases could explain the eventual alleviation of patient complaints.

Our studies showed that following the substitution of methadone (2 mg/kg/inj) for morphine self-injection in the dependent rat, interinjection intervals gradually became shorter until they stabilized at 1–2 hr [32]. Following substitution of LAAM (1 mg/kg/inj) for morphine or methadone, rats took several LAAM injections within a 3 hr span, suggesting the lack of immediate reinforcement. The next LAAM self-injection did not occur until 25–30 hr later, presumably due to the production of active LAAM metabolites. Thereafter, single or double LAAM self-injections occurred on a more regular basis. On the other hand, after substitution of NLAAM (1 mg/kg/inj) for morphine or methadone, rats immediately began to self-administer NLAAM at relatively regular intervals. There was no transitional effect as observed following LAAM substitution. These data further define unique pharmacokinetic properties of LAAM. Our experimental data suggest that establishment of LAAM maintenance in humans might be less traumatic if initial doses consisted of a mixture of LAAM and NLAAM.

Self-administration of opioid-like peptides.

DYN and D-ala²-DYN. Dynorphin-(1–13) (DYN) is a potent endogenous opioid-like peptide originally isolated from porcine hypophysis and intestine [10,25]. DYN has been shown to possess specific binding properties for *kappa* receptors [6,9] and has been found to suppress withdrawal symptoms in morphine-dependent monkeys [1]. We explored the possibility that DYN might be self-administered by morphine-dependent rats.

Upon stabilization of morphine self-administration (10 mg/kg/inj) at a FR-10, DYN or D-ala²-dynorphin-(1–11) (D-ala²-DYN) at a dose of 125 or 250 μg/kg/inj was substituted for morphine [18]. Rats self-administered these opioid peptides at both dose levels. Self-injection patterns of DYN demonstrated shorter interinjection intervals compared to morphine, and, thus, reflected a shorter acting agent.

D-enkephalin. D-enkephalin (10 mg/kg/inj) was also found to substitute for morphine self-injection (10 mg/kg/inj) in dependent rats. A shift to shorter interinjection intervals emerged, resulting in a two-fold increase in the total number of injections taken per 24 hr [27]. These findings demonstrated that D-enkephalin, like many other opioids, substituted for morphine and served as a reinforcer of schedule-controlled behavior.

Extinction (saline substitution) of opioid self-administration. When saline was substituted for morphine, methadone, LAAM, NLAAM or DNLAAM in self-administering rats, significant increases in head-shake behavior occurred during morphine, methadone, NLAAM and DNLAAM abstinence, but not during LAAM abstinence [35]. Furthermore, we have demonstrated that naloxone-induced abstinence in morphine- and LAAM-dependent rats resulted in similar withdrawal symptoms [34]. Thus, the relatively mild abstinence syndrome occurring after LAAM withdrawal is most likely related to the relatively long plasma half-lives of the two pharmacodynamically active N-demethylated LAAM metabolites, NLAAM and DNLAAM.

Pharmacodynamic differences between the effects of *kappa* opioid withdrawal (KC, EKC, DYN, D-ala²-DYN) and morphine withdrawal have also been shown. Saline substitution for *kappa* agonists during self-administration resulted in increases in saline self-injection rates over opioid self-injection rates [18,31]. However, saline substitution did not result in the emergence of a morphine-like abstinence syndrome. Symptoms such as diarrhea, ptosis and head shakes were minimal compared to those seen during morphine abstinence. Moreover, occurrences of sleep and REM sleep were not disrupted. These data suggest that chronic administration of *kappa* opioids produces a relatively lower degree of physical dependence.

Relapse to Opioid Self-Administration in Morphine Post-Addict Rats

Pharmacodynamic blockade by subcutaneous naloxone pellets. We investigated the effects of subcutaneously implanted placebo and naloxone pellets on the relapse to morphine self-administration in post-addict rats. Post-addict rats implanted with placebo pellets and allowed to self-administer morphine relapsed to their previous levels of morphine self-administration. However, rats implanted with two 100 mg naloxone pellets extinguished their drug-seeking behavior [20,36]. As expected, EEG recordings revealed that naloxone treatment also blocked morphine-induced EEG slow-wave bursts. Similar correlations between EEG and behavior have also been reported in human subjects. The administration of naloxone antagonized the subjective and EEG effects of heroin challenges in post-addict volunteers [28].

Relapse to mixed agonist-antagonists. In a recent review,

the self-administration of several mixed opioid agonist-antagonists was assessed [3]. Clinical studies have demonstrated that pentazocine abuse is more predominant in humans with histories of prior opioid abuse [2, 5, 22]. In our studies, we assessed the abuse potentials of mixed agonist-antagonists in morphine post-addict rats.

When morphine post-addict rats were given unlimited access to self-administration of morphine, the mean daily number of self-injections stabilized at an average of 13 self-injections per 24 hr by the seventh day [24]. During relapse to the self-administration of pentazocine, nalbuphine, and butorphanol, the mean daily number of self-injections similarly increased over the seven days of the relapse period studied. In contrast, a pattern of extinction emerged when morphine post-addict rats were allowed to self-administer saline. That is, the mean number of saline self-injections was very high on the first day of relapse, then steadily declined.

During the substitution of saline for the above opioids, lever pressing patterns of extinction emerged. During saline substitution, mean daily total REM sleep times were significantly suppressed in morphine- and to a lesser extent in pentazocine-dependent rats, but not in nalbuphine- and butorphanol-dependent rats. The degree of REM sleep suppression has been considered to be a measure of severity of withdrawal from opioids [35]. It was, therefore, suggested that considerable differences in the pharmacodynamics of these opioids exist. A relatively high degree of physical dependence existed in rats self-administering morphine, a moderate degree with pentazocine, and a relatively lower degree of physical dependence prevailed during butorphanol and nalbuphine self-administration.

SUMMARY AND CONCLUSIONS

Our experimental rat model of addiction, which allows continuous recording of EEG and EMG activities and programming of intravenous drug injections, has been used to study the self-administration of several opioids. Comparative data on self-injection patterns during the self-administration of morphine, methadone, LAAM, LAAM metabolites, *kappa* opioids and opioid peptides demonstrated similarities and differences in potencies and durations of action. The study of the associated EEG and behavioral correlates of opioid self-administration contributed to the delineation of pharmacodynamic and pharmacokinetic characteristics of opioids. For example, unique pharmacodynamic and pharmacokinetic characteristics of LAAM were attributed to its active N-demethylated metabolites, NLAAM and DNLAAM. Pharmacodynamic differences between the effects of *kappa* opioid withdrawal and morphine withdrawal on EEG and behavior were demonstrated. The ability of subcutaneous naloxone pellets to prevent relapse to morphine self-administration and to block morphine-induced EEG slow-wave bursts in post-addict rats was shown. The mixed agonist-antagonists pentazocine, nalbuphine and butorphanol were self-administered by morphine post-addict rats. However, EEG and behavioral data indicated that relatively lower degrees of physical dependence existed in rats self-administering these mixed agonist-antagonists than with morphine.

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