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Comparison of Abstinence Syndromes Following Chronic Administration of Mu and Kappa Opioid Agonists in the Rat[1]

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YOUNG, G. A. AND N. KHAZAN. *Comparison of abstinence syndromes following chronic administration of mu and kappa opioid agonists in the rat.* PHARMACOL BIOCHEM BEHAV **23**(3) 457–460, 1985.—Abstinent states were compared following chronic administration of *mu* and *kappa* opioid agonists, morphine and ethylketocyclazocine, respectively. Rats were prepared with chronic EEG and EMG electrodes and indwelling IV cannulae. One group of rats was chronically administered IV morphine, while a second group received chronic injections of IV ethylketocyclazocine. Morphine abstinence was associated with suppression of REM sleep occurrences, increases in number of wet-dog shakes, and a decline in EEG spectral power during slow-wave sleep episodes. In contrast, the ethylketocyclazocine abstinence syndrome included minor abstinence signs. Differences in abstinent states between morphine and ethylketocyclazocine indicate the involvement of separate receptor populations in the process of dependence on morphine and ethylketocyclazocine.

Morphine Ethylketocyclazocine Abstinence syndrome

WE have previously assessed and compared several pharmacodynamic properties of *mu* and *kappa* opioid agonists in the rat. In acute experiments, intravenously administered morphine (*mu* opioid agonist) to freely moving rats, bearing chronic cortical EEG and temporalis muscle EMG electrodes and IV cannulae, produced high-voltage cortical EEG slow-wave bursts that were associated with dose-related increases in EEG spectral power in the zero to 10 Hz range [19]. Ketocyclazocine (*kappa* opioid agonist) was found to produce high-voltage cortical EEG slow-wave bursts associated with a predominant spectral peak in the 5–8 Hz band. In both cases, high-voltage EEG slow-wave bursts were associated with behavioral stupor and exopthalmos. The acute opioid EEG and behavioral effects of methadone (*mu* opioid agonist) and ketocyclazocine have been shown to be stereospecific [18]. The (−) enantiomers were found to produce the opioid EEG characteristics and the (+) enantiomers to be inactive. In addition, acute effects of morphine on EEG and behavior were antagonized by lower doses of naloxone than those needed to antagonize ketocyclazocine effects [18].

In a chronic experiment with repeated injections of morphine and ethylketocyclazocine, tolerance developed to effects on EEG and behavior [17]. In both cases, the intensity of EEG slow-wave bursting and behavioral stupor diminished as reflected by significant quantitative reductions in EEG power spectral densities. In a cross-tolerance study, ethylketocyclazocine-tolerant rats were found to be cross-tolerant to the EEG and behavioral effects of morphine [17]. However, no cross-tolerance to effects of ethylketocyclazocine in morphine-tolerant rats was observed.

Preliminary observations in our laboratory indicated that while withdrawal of morphine in morphine-tolerant rats was associated with a severe abstinence syndrome that included occurrences of wet-dog shakes, diarrhea, ptosis, abdominal stretching and irritability; withdrawal of ethylketocyclazocine in ethylketocyclazocine-tolerant rats was associated with a milder abstinence syndrome. In an attempt to substantiate these findings, we have further evaluated and compared three EEG and behavioral parameters during the states of morphine and ethylketocyclazocine withdrawal. First, we assessed the distribution and amount of rapid eye movement (REM) sleep episodes occurring during withdrawal from morphine and ethylketocyclazocine. We have previously demonstrated that the degree and time course of REM sleep suppression during morphine and methadone abstinence coincided with occurrences of withdrawal signs and symptoms [20, 21, 22]. Thus, degree of suppression in REM sleep provides an index of the severity and duration of an opioid abstinence syndrome. Second, we assessed and compared increases in wet-dog shakes during withdrawal from morphine and ethylketocyclazocine. Wet-dog shakes during morphine abstinence in the rat is a well-established behavioral correlate [2,11]. Third, we determined the EEG power spectral densities associated with slow-wave sleep episodes during withdrawal from morphine and ethylketocyclazocine. It has been previously shown that morphine withdrawal in the rat is associated with a decline in

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EEG integrated voltage output during the behavioral states of wakefulness and slow-wave sleep [5].

METHOD

Sixteen female Sprague-Dawley rats (250–300 g) were implanted with bipolar epidural frontoparietal EEG and temporalis muscle EMG recording electrodes and with chronic cannulae in the jugular vein. Surgical procedures have been previously described [4]. Intravenous cannulae were prepared and implanted according to the method of Weeks [13,14]. During the experiments, the rats were housed in individual cages (12×12×24 in.). To permit free movement of the rat, each cage was equipped with a swivel connector having concentric mercury pools which served as noise-free sliding contacts [7]. These freely-moving rats were allowed to acclimatize to the experimental cages for two to three days before experimentation. Lighting conditions consisted of a timer-regulated lights-off period from 10 p.m. to 6 a.m.

For each rat, direct EEG activity was filtered to pass frequencies between 1 and 35 Hz. The EEG and integrated EMG activity were continuously recorded on a Grass polygraph. The EEG was simultaneously recorded on FM magnetic tape using a Hewlett-Packard Model 3940-A recorder. Power spectral analysis of EEG was performed offline using a Nicolet MED-80 minicomputer system; EEG power spectra were derived from 10-sec samples of EEG that were digitized at a sampling rate of 100/sec, and power spectral densities were estimated at 0.05 Hz intervals from 0 to 50 Hz [6,23]. Average power spectra were obtained by averaging spectra derived from six 10-sec epochs; weighted geometric smoothing over three neighboring frequencies was used.

Behavioral states of sleep, REM sleep and wakefulness were identified by corresponding changes in EEG and EMG recordings [4,7]. 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 pen deflections [2,21]. These head-shakes were usually a component of "wet-dog shakes" [11].

Eight rats were treated chronically with morphine by a series of automatic intravenous injections. Morphine sulfate was dissolved in isotonic saline at a concentration of 10 mg/ml. On the first day, the rats received hourly injections of 1.25 mg/kg of morphine. The dose was then increased to 2.5, 5.0 and 10.0 mg/kg/hr on successive days. On the fifth day, the rats received 10.0 mg/kg of morphine every 3 hr and were subsequently maintained on this schedule of injections through eight days. On the ninth day morphine was withdrawn and replaced by saline. Effects of morphine withdrawal on the distribution and amount of REM sleep, distribution and amount of wet-dog shakes, and slow-wave sleep EEG power spectra were assessed.

A second group of eight rats was chronically treated with ethylketocyclazocine by a series of automatic intravenous injections. Ethylketocyclazocine methanesulfonate was dissolved in a minimal amount of 0.5 N NaOH and brought up to a concentration of 2.5 mg/ml with isotonic saline. On the first day, the rats received a dose of 2.5 mg/kg 2 hr. The dose was then increased to 5.0 and 10.0 mg/kg per 2 hr every other day with 10.0 mg/kg/2 hr being the final dose. On the ninth day saline was substituted for ethylketocyclazocine. The same experimental parameters were evaluated in this group of withdrawn rats; namely, amount and distribution of REM sleep, amount and distribution of wet-dog shakes and slow-wave sleep EEG power spectra.

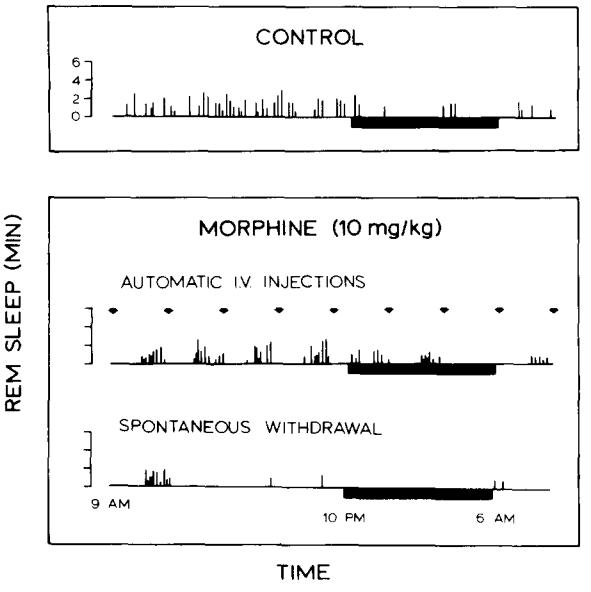

FIG. 1. REM sleep distribution in the rat for a control day and for the last day of chronic morphine administration and the first day of spontaneous withdrawal. Automatic IV injections of morphine are indicated by the arrows. The timer-regulated period of darkness is indicated on the TIME scale. Note the drastic suppression of REM sleep episode upon spontaneous morphine withdrawal.

RESULTS

Our chronic IV dose schedules used for morphine and ethylketocyclazocine in the present study resulted in a state of tolerance to EEG and behavioral effects. During the first 24 hr of morphine withdrawal in the morphine-tolerant rats, an average of 17.00±5.3 (mean±S.D.) min of REM sleep occurred. However, an average of 89.7±23.1 min of REM sleep occurred in the ethylketocyclazocine-tolerant rats. An analysis of variance indicated that the mean amounts of REM sleep during the first 24 hr of spontaneous withdrawal in the morphine and ethylketocyclazocine groups were significantly different, $F(1,14)=75.77$, $p<0.001$. As shown in more detail in Fig. 1, during the first 24 hr of morphine withdrawal in a representative morphine-tolerant rat, occurrences of REM sleep were severely suppressed. In contrast, as illustrated in Fig. 2 for a representative ethylketocyclazocine-tolerant rat, during the first 24 hr of withdrawal occurrences of REM sleep were not suppressed. Furthermore, administration of the opioid antagonist naltrexone (2 mg/kg, PO) to ethylketocyclazocine-tolerant rats had no effect on REM sleep occurrences.

During morphine and ethylketocyclazocine withdrawal similar increases in wet-dog shakes ($p>0.20$) were seen over the first 24 hr (Fig. 3). It should be emphasized that increases in wet-dog shakes appeared to be the only behavioral change that occurred during both morphine and ethylketocyclazocine withdrawal.

As demonstrated in Fig. 4, total slow-wave sleep EEG spectral power over the zero to 20 Hz range averaged 27.75±6.20 (mean±S.D.) μV^2/Hz during morphine dependence and 16.85±4.85 μV^2/Hz during the 8th hr of morphine withdrawal. An analysis of variance indicated that this difference in mean EEG spectral power was significant,

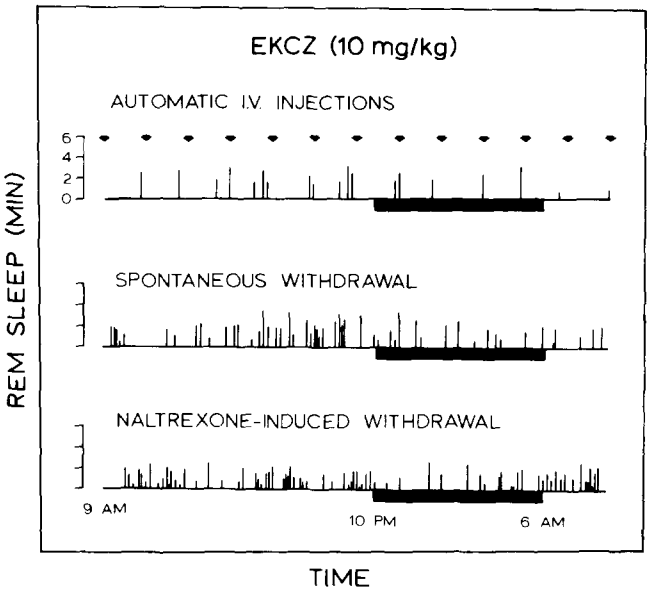

FIG. 2. REM sleep distributions for the last day of chronic ethylketocyclazocine administration, the first day of spontaneous withdrawal and the first day of naltrexone-induced withdrawal. Automatic IV injections of ethylketocyclazocine are indicated by the arrows. The timer-regulated period of darkness is indicated on the TIME scale. Note the lack of an effect on REM sleep upon spontaneous as well as naltrexone-induced withdrawal.

$F(1,14)=15.29$, $p<0.005$. In the case of ethylketocyclazocine, total mean slow-wave sleep EEG spectral power averaged 28.85 ± 7.50 $\mu V^2/Hz$ during dependence and 26.20 ± 3.16 $\mu V^2/Hz$ during abstinence. This difference in mean total EEG spectral power was not statistically significant ($p>0.20$).

DISCUSSION

Few comparative studies dealing with opioid abstinence have been reported [9]. When cyclazocine, a benzomorphan with purported kappa and sigma agonistic effects and mu antagonistic properties, was chronically administered to the monkey, large doses of naloxone precipitated a mild abstinence syndrome [12]. In the chronic spinal dog preparation, morphine and cyclazocine abstinence syndromes were found to be qualitatively different [3]. Similarly, in man the cyclazocine abstinence syndrome was found to be milder and qualitatively different than the morphine abstinence syndrome [10].

Our present results demonstrated that withdrawal of ethylketocyclazocine in ethylketocyclazocine-tolerant rats was associated with minimal abstinence signs. A significant behavioral change during ethylketocyclazocine withdrawal was increases in wet-dog shakes. In a previous study we observed that when morphine-dependent rats self-administered 10 mg/kg IV injections of morphine every two to three hr, lever pressing behavior was preceded by increases in wet-dog shakes [1]. Other behavioral changes such as ptosis and diarrhea were not evident during the interinjection intervals. Thus, it appears that wet-dog shakes emerge early during morphine-seeking behavior and are part of an early abstinence state, and that all other signs of withdrawal such as ptosis and diarrhea emerge later. We also

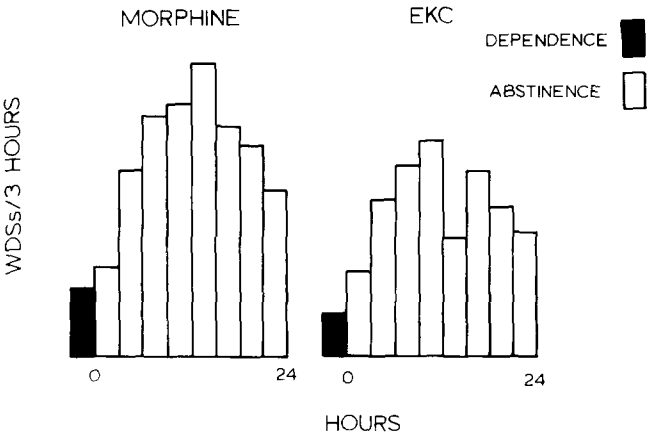

FIG. 3. Mean number of wet-dog shakes are shown as a function of successive 3-hr periods during spontaneous abstinence from morphine and ehtylketocyclazocine.

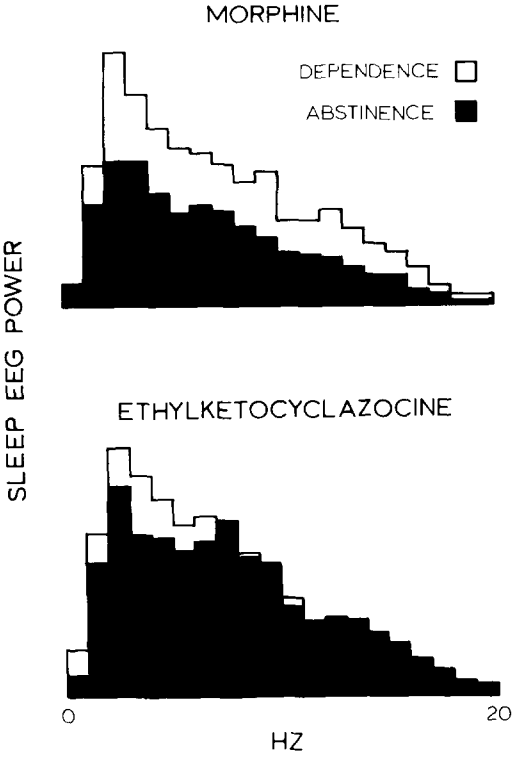

FIG. 4. Mean Sleep EEG power spectra are shown during dependence on morphine and ethylketocyclazocine and during the 8th hr of spontaneous abstinence. Sleep EEG power is presented as a function of 1 Hz intervals.

found similar abstinence syndromes with other kappa opioid agonists in self-administration studies, when saline was substituted for the benzomorphans ketocyclazocine and ethylketocyclazocine [16], and for the purported kappa opioid peptide dynorphin-[1–13] and its synthetic long-acting analogue D-ala^2-dynorphin-[1–11] [8]. Primary dependence studies in the monkey also have indicated that several benzomorphans possess *low physical* dependence liability [15].

In contrast, morphine withdrawal is associated with a severe abstinence syndrome [20, 21, 22].

In general, then, the results demonstrate several differences between the pharmacodynamic properties of morphine and ethylketocyclazocine. Acute morphine injections have been found to produce characteristic EEG and EEG power spectral effects that are antagonized by relatively low doses of naloxone [18,19]. Chronic morphine injections induce tolerance, but no cross-tolerance to ethylketocyclazocine [17]. Physical dependence on morphine is established as reflected by the emergence of many abstinence signs during morphine withdrawal. In contrast, acute ethylketocyclazocine injections produced qualitatively different EEG and EEG power spectral effects from those produced by morphine [19]. Relatively more naloxone is required to antagonize these acute effects of ethylketocyclazocine [18]. Chronic ethylketocyclazocine injections induce tolerance to ethylketocyclazocine and cross-tolerance to EEG and behavioral effects of morphine [17]. Finally, withdrawal of ethylketocyclazocine in ethylketocyclazocine-tolerant rats is associated with the emergence of minimal abstinence signs. These data suggest the involvement of different opioid receptor populations in the pharmacodynamics associated with the administration of *mu* and *kappa* opioid agonists.

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