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Sleep-Inducing Effects of Three Hypnotics in a New Model of Insomnia in Rats

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HALPERIN, J. M., D. MILLER AND L. C. IORIO. *Sleep-inducing effects of three hypnotics in a new model of insomnia in rats.* PHARMAC. BIOCHEM. BEHAV. 14(6) 811–814, 1981—Sleep-inducing effects of hypnotic drugs are difficult to demonstrate in rats because of high baseline sleep times. Most increases in slow wave sleep (SWS) following the administration of hypnotics have been found to be at the expense of REM sleep rather than waking. In the first experiment we found that rats chronically implanted with electrodes for recording ECoG and EMG slept significantly less during the first two hours of the dark period when housed under 16-hr light 8-hr dark (16L/8D) than when housed under 12L/12D conditions. The second experiment examined the effects of flurazepam, phenobarbital and thalidomide administered orally at the onset of the two-hour period of increased waking resulting from the 16L/8D lighting. All three drugs caused dose-dependent reductions in waking and increases in SWS with no alterations in REM% of total sleep time. Sleep onset latency was also significantly reduced by all three drugs. As in man, flurazepam was the most potent, and thalidomide was the least potent of the hypnotics in rats.

Hypnotics Sleep Light/dark cycle Flurazepam Phenobarbital Thalidomide Rats

THE PRECLINICAL evaluation of novel compounds for hypnotic potential usually consists of at least one relatively simple procedure to evaluate behavioral and/or central nervous system depression [2, 4, 7, 10], followed by direct measurements of sleep using polygraphic techniques [1]. Polygraphic evaluation is most frequently performed on either rats or small primates. The evaluation of hypnotics in some primates has been reported to yield data which is highly predictive of human hypnotic potency [1]. However, studies using rats have been less successful. Rats normally spend more than half of the time sleeping [11], thus making it difficult to significantly increase their sleep time with hypnotics. Most studies examining the effect of hypnotics on sleep in rats have used very high doses in order to show increases in slow wave sleep (SWS). These increases have usually been at the expense of rapid eye movement (REM) sleep, rather than waking [1, 9, see [10] for review]. Furthermore, increasing "normal" sleep levels may be quite different than raising decreased levels of sleep, as in insomniacs, to normal levels.

More recently, researchers have attempted to produce rat models of insomnia that would be more sensitive to the sleep-inducing effects of drugs. Attempts to reduce sleep times in rats, however, have all involved intrusive manipulations such as periodic electric shocks [3] or forced motor activity [8]. We found that by lengthening the light phase of the light/dark cycle, a period of relative insomnia can be produced in rats which is reversible by different standard hypnotics at doses lower than those previously reported.

GENERAL METHOD

Male Sprague Dawley albino rats (250–300 g) were anesthetized with Equithesin and chronically implanted with bipolar stainless steel electrodes for recording electrocorticogram (ECoG) and electromyogram (EMG). Throughout the experiments all rats were housed indicvidually and had access to food and water ad lib.

ECoG and EMG were recorded from subjects on a Grass model 78D polygraph at a paper speed of 5 mm/sec. Sleep states were scored in 15 sec epochs according to the following criteria: Waking was defined as rapid, low voltage ECoG in conjunction with high voltage EMG; SWS was defined as high voltage, low frequency (1–4 Hz) ECoG along with reduced muscle tone; and REM sleep was characterized by the presence of theta waves (6–9 Hz) in the ECoG and muscle atonia. All polygraphic records were scored blind.

EXPERIMENT 1

This model of insomnia is based on the observation that rats housed under 12-hr light/12-hr dark (12L/12D) lighting conditions are most active and engage in most goal-oriented behaviors (e.g., grooming or feeding) in the dark, while these behaviors appear to be suppressed during the light period [5,6]. We hypothesized that an extension of the light period would result in an increased duration of the suppression of the goal-oriented behaviors, thus increasing the motivation to engage in them at the onset of the dark period. The

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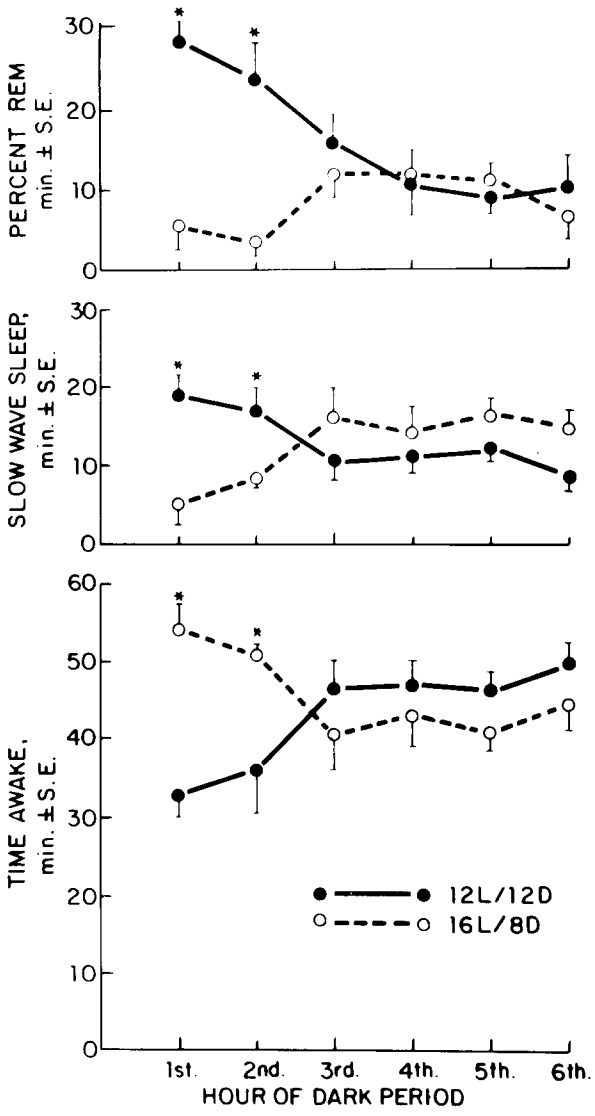

FIG. 1. The mean minutes awake, minutes of SWS, and REM% of total sleep time for rats housed under 12L/12D and 16L/8D lighting during the first six hours into the dark period. Vertical bars represent SE. *$p<0.05$.

enhancement of these goal-oriented behaviors should result in a period of time during which the rats are more active and minimal sleep occurs, thus creating a period of "insomnia" in rats while under a minimal amount of stress.

METHOD

Six rats were maintained under a reverse 12L/12D cycle such that the room was dark from 0800–2000. After four weeks adaptation to this light/dark cycle they were connected to the recording apparatus for a 24-hr adaptation period. ECoG and EMG were recorded during each of the next three days for a 6-hr period beginning at the onset of the dark cycle. At the end of the third recording session the rats were disconnected from the recording apparatus and the lighting was altered to a 16L/8D cycle so that the dark period terminated at 1600; the onset of the dark period remained unaltered. After 7–10 days of adaptation to this new cycle the rats were reconnected to the recording leads, allowed 24-hr adaptation, and the ECoG and EMG were recorded for three more 6-hr sessions beginning at the onset of the dark period.

Comparisons were made between the two lighting conditions for changes in waking, SWS, REM percent of total sleep time (REM%) and sleep onset latency using a correlated t-test. Sleep onset was defined as four consecutive epochs of sleep.

RESULTS

As shown in Fig. 1, rats remained awake significantly longer during the first two hours of the dark period when housed under 16L/8D conditions as compared to 12L/12D conditions. This increased waking was followed by a tendency toward increased sleep during the subsequent hours. The insomnia occurring during each of the two hours following the onset of the dark period was characterized by a significant decrease in both SWS and REM%. Sleep patterns of rats obtained as long as six weeks after the change in lighting cycle showed no diminution in the amount of waking during the first two hours of the dark period.

DISCUSSION

The data show that the lengthening of the light cycle to 16L/8D resulted in a period of relative insomnia in rats. The cause of this period of increased waking is not certain; however, it is probably due to an increase in behaviors that are normally suppressed during the light period. Irrespective of its cause, this period of increased waking appears to be less stressful than those previously described and may be useful for the evaluation of hypnotics in rats.

EXPERIMENT 2

This study examined whether the increased wakefulness resulting from 16L/8D lighting is readily reversible by standard hypnotics, thus making it useful for the evaluation of novel compounds. Three hypnotics from different chemical classes were used to evaluate whether this model of insomnia might be useful over a wide range of novel compounds.

METHOD

Thirty six chronically implanted rats that were housed under 16L/8D lighting conditions for a minimum of three weeks were tested using the following three-day paradigm: 24 hr adaptation to the recording leads, vehicle injection on day 2, drug injection on day 3.

All drugs were suspended in a 0.4% methylcellulose solution and were administered orally in a volume of 1 ml/kg at the onset of the dark period. The drugs evaluated were flurazepam (6.25, 12.5, 25.0 and 50.0 mg/kg), phenobarbital (6.25, 12.5, 25.0, 50.0 and 100.00 mg/kg), and thalidomide(12.5, 25.0, 50.0 and 100 mg/kg). Each dose of each drug was tested in six rats. No rat received more than six drug injections and all drug injections were separated by at least one week.

RESULTS

All three drugs caused dose-dependent reductions in time awake and increases in SWS. Flurazepam significantly re-

TABLE 1

EFFECTS OF FLURAZEPAM ON SLEEP IN RATS DURING A 120-MINUTE RECORDING SESSION

Dose, mg/kg PO	n	Mean time awake min (SE)	Mean SWS min (SE)	Mean REM % (SE)	Mean sleep onset latency, min (SE)
Vehicle	24	100.1 (2.0)	18.4 (1.8)	5.8 (1.4)	47.7 (4.8)
6.25	6	105.8 (1.2)	13.5 (1.4)	6.1 (1.8)	42.1 (9.2)
12.5	6	90.7 (5.3)*	28.6 (5.1)*	2.3 (0.5)	21.1 (4.0)†
25.0	6	80.0 (4.9)‡	38.9 (4.9)‡	3.1 (1.2)	21.7 (9.2)*
50.0	6	71.5 (7.1)‡	46.9 (7.0)‡	3.5 (1.7)	16.3 (3.0)‡

*Significantly different from vehicle control, $p<0.05$.
†Significantly different from vehicle control, $p<0.01$.
‡Significantly different from vehicle control, $p<0.005$.

TABLE 2

EFFECTS OF PHENOBARBITAL ON SLEEP IN RATS DURING A 120-MINUTE RECORDING SESSION

Dose, mg/kg PO	n	Mean time awake min (SE)	Mean SWS min (SE)	Mean REM % (SE)	Mean sleep onset latency, min (SE)
Vehicle	30	99.1 (2.0)	19.5 (1.7)	5.2 (1.1)	49.8 (5.2)
6.25	6	99.6 (3.4)	19.3 (2.9)	3.6 (2.3)	32.5 (7.6)
12.5	6	95.0 (2.1)	24.1 (1.8)	3.4 (1.9)	23.5 (7.8)*
25.0	6	81.7 (4.1)†	36.3 (4.0)†	5.3 (2.2)	33.8 (4.3)
50.0	6	70.0 (4.7)†	48.3 (4.1)†	3.0 (1.4)	24.8 (4.3)*
100.0	6	59.8 (7.7)†	54.2 (7.4)†	6.8 (4.2)	15.5 (2.5)†

*Significantly different from vehicle control, $p<0.05$.
†Significantly different from vehicle control, $p<0.005$.

TABLE 3

EFFECTS OF THALIDOMIDE ON SLEEP IN RATS DURING A 120-MINUTE RECORDING SESSION

Dose, mg/kg PO	n	Mean time awake min (SE)	Mean SWS min (SE)	Mean REM % (SE)	Mean sleep onset latency, min (SE)
Vehicle	24	98.8 (2.8)	18.6 (2.3)	7.3 (1.7)	50.8 (6.9)
12.5	6	106.4 (2.5)	12.3 (2.1)	8.0 (3.2)	51.9 (16.0)
25.0	6	88.7 (4.2)	29.2 (3.6)*	6.1 (2.3)	23.5 (5.5)*
50.0	6	85.3 (5.8)*	33.5 (5.8)†	3.6 (1.7)	30.3 (10.3)
100.0	6	69.4 (4.9)†	49.0 (4.9)†	3.3 (1.0)	20.6 (3.0)*

*Significantly different from vehicle control, $p<0.05$.
†Significantly different from vehicle control, $p<0.005$.

duced waking time and sleep onset latency, and increased slow wave sleep at 12.5 mg/kg and higher doses. REM% of total sleep time was not altered significantly (See Table 1).

Table 2 shows the effects of phenobarbital on the sleep of rats. At doses of 25.0 mg/kg and higher, waking was significantly reduced while SWS was significantly increased. REM% was not significantly altered. Sleep onset latency was reduced significantly at 12.5, 50.0 and 100.0 mg/kg, but not at 25.0 mg/kg.

As shown in Table 3, thalidomide significantly increased SWS at 25 mg/kg and higher and decreased waking at doses of 50.0 mg/kg and higher. Sleep onset latency was reduced at 25.0 and 100 mg/kg. REM% appeared to be decreased by thalidomide in a dose-dependent manner, however these effects were not significant.

DISCUSSION

All three drugs significantly increased slow wave sleep and decreased waking in a dose-dependent manner. As in

man, flurazepam was the most, and thalidomide was the least potent.

GENERAL DISCUSSION

We have described and evaluated a model of insomnia in rats. This model yields a 2-hr period during which rats sleep a minimal amount of time without intrusive manipulations. This period of relative insomnia was readily reversible by three different hypnotic drugs such that the increased sleep was at the expense of waking, rather than REM sleep. Furthermore, these alterations in sleep occurred at doses lower than those previously reported [1,3].

In summary, rats housed under 16L/8D lighting conditions have minimal amounts of sleep during the first two hours of the dark period. This increased waking is readily reversed by standard hypnotics and is advantageous for the evaluation of hypnotic potential in novel compounds.

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