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Phenoxybenzamine and Bromocriptine Attenuate Need for REM Sleep in Rats

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RADULOVACKI, M., W. J. WOJCIK, R. WALOVITCH AND M. BRODIE. *Phenoxybenzamine and bromocriptine attenuate need for REM sleep in rats.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 371–375, 1981.—Phenoxybenzamine (10 mg/kg, IP), an α-adrenoreceptor blocker, and bromocriptine (5 mg/kg, IP), a dopamine receptor stimulant, were administered to rats while the animals were being deprived of REM sleep by selective REM sleep deprivation method. We have shown recently that α-adrenoreceptor blockers and bromocriptine when administered to rats after the animals had been deprived of REM sleep were able to abolish REM sleep rebound and thus attenuate the need for REM sleep. The purpose of this study was to investigate whether these agents might also have the capacity to attenuate the need for REM sleep when given to animals in a situation when the need for REM sleep is being generated, i.e. during REM sleep deprivation. Our results show that administration of phenoxybenzamine or bromocriptine to rats immediately before or during the period of REM sleep deprivation also abolished appearance of subsequent REM sleep rebound. This suggests that administration of the two pharmacological agents prevented the generation of REM sleep pressure by fulfilling the need for REM sleep.

Phenoxybenzamine Bromocriptine REM sleep need Rats

RAPID eye movement (REM) sleep rebound, or recovery, represents a compensatory increase in REM sleep time and is only to a certain extent proportional to the amount of REM sleep lost during the REM sleep deprivation period [6]. It also reflects an increased need for REM sleep in animals that were deprived of it. Recently, we reported that the administration of two α-adrenoreceptor blockers, phentolamine and phenoxybenzamine [8], and a dopamine agonist, bromocriptine [3], to rats deprived of REM sleep, eliminated REM sleep rebound in those animals [9,10]. Since α-adrenoreceptor blockers and bromocriptine, administered to rats after the animals had been deprived of REM sleep, were able to abolish REM sleep rebound and thus attenuate the need for REM sleep, these agents might also be expected to have the capacity to attenuate the need for REM sleep when given to animals in a situation when the need for REM sleep is being generated, i.e. during REM sleep deprivation.

In order to test this possibility we administered phenoxybenzamine and bromocriptine to rats while the animals were being deprived of REM sleep by a selective REM sleep deprivation method [6], so that the drug action occurs during the REM sleep deprivation period. Our results indicate that administration of the two pharmacological agents prevented the generation of REM sleep need since no REM sleep rebound appeared in those drug treated animals.

METHOD

Experiment 1

Adult male Sprague-Dawley rats (350–400 g) 4 months old were implanted under pentobarbital anesthesia (40 mg/kg) with cortical and dorsal neck muscle electrodes for polygraphic recording. One week after surgery, animals were selectively deprived of REM sleep for twenty-four hours by the "flower pot" technique [6]. To standardize the degree of REM sleep deprivation (RD), rats were placed on circular platforms whose surface area corresponded to their body weight [5,7]. A surface area to animal weight ratio of 14 cm^2/100 g was used. The same parameters were used in our previous study which clearly showed that by using the flower pot a selective deprivation of REM sleep can be obtained in rats [9], although there had been some reservations concerning the use of this technique in the past [1]. During 24 hours of RD, animals were divided into three groups. The animals in the first group received drug vehicle (propylene glycol, 0.5 ml/kg, IP) and served as the RD control group. Those in the second group received phenoxybenzamine (10 mg/kg, IP) and the rats in the third group received bromocriptine (5 mg/kg, IP). In the fourth group, animals were housed in their cages and served as a non-RD control group. They received drug vehicle as the RD control group. Drugs and drug vehicle were given according to the schedule described in Table 1.

Rationale for the drug schedule was based on our previous experience with administration of phenoxybenzamine and bromocriptine to RD rats when a dose of 10 mg/kg phenoxybenzamine eliminated REM sleep rebound for 18 hours and a dose of 5 mg/kg bromocriptine abolished REM sleep rebound for 6 hours [9,10]. Therefore, in the present study phenoxybenzamine was administered only once (18 hours before the animals were taken off RD) while bromo-

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TABLE 1

DRUG SCHEDULE FOR EXPERIMENT 1

Treatment	RD Begins 8 a.m.	2 p.m.	8 p.m.	2 a.m.
· Drug vehicle (non-RD control)	·	·	·	·
· Drug vehicle (RD control)	·	·	·	·
* Phenoxybenzamine, 10 mg/kg	·	*	·	·
† Bromocriptine, 5 mg/kg	†	†	†	†

criptine was given four times (the last injection was given 6 hours before the animals were taken off RD) under the assumption that the drugs would cover most of the 24 hour RD period and thus prevent the generation of REM sleep "need".

Experiment 2

This experiment was done in the same way as Experiment 1 except the animals were deprived of REM sleep for 48 hours and were polygraphically recorded for sixty continuous hours following RD. One group of animals received phenoxybenzamine (10 mg/kg, IP) immediately prior to RD while the other group received the drug vehicle (propylene glycol, 0.5 ml/kg, IP) at the same time and served as RD control.

Evaluation of polygraphic record from Experiments 1 and 2 was made using standard techniques where each epoch of record was determined to be either wakefulness, slow wave sleep (SWS) or REM sleep. The epochs were 50 seconds long and the speed of the paper drive was 100 sec/page of paper.

Statistics for Experiment 1 were performed on the total time each treatment affected wakefulness, SWS or REM sleep for the first 24 hours after RD and for the first 30 hours after RD for the Experiment 2. These time periods were selected because both contain REM sleep rebound periods of the corresponding RD control groups. Statistics were then performed on the remainder of the polygraphic recording, i.e., for the 24–36 hour period (Experiment 1) or for the 30–60 hour period (Experiment 2). These time periods contained normal REM sleep that followed the REM sleep rebound of the corresponding RD control groups. The statistical test made was the one-way analysis of variance (ANOVA) with comparisons made to the non-RD control groups by the Scheffe test. Statistical comparison for REM sleep during the 0.30 hour time period in Experiment 2 was performed by Steel's non-parametric test with comparisons to non-RD control. Also, a two-way ANOVA was performed on REM sleep for the entire recording session. The recording was divided into six 6 hour intervals for the Experiment 1 and ten 6 hour intervals for the Experiment 2. Comparisons to the non-RD control group were made by the Newman-Keuls test.

TABLE 2

EFFECTS OF BROMOCRIPTINE (5 mg/kg, IP) AND PHENOXYBENZAMINE (10 mg/kg, IP) ON WAKEFULNESS, SWS AND REM SLEEP IN RATS DURING THE FIRST 24 HR AFTER REM SLEEP DEPRIVATION. DRUGS WERE GIVEN DURING REM SLEEP DEPRIVATION

	N	Wakefulness	SWS	REM Sleep
Non-RD Control	5	616 ± 40	698 ± 43	130 ± 10
RD Control	8	485 ± 18†	726 ± 20	228 ± 18‡
Bromocriptine	8	593 ± 67	697 ± 51	150 ± 20
Phenoxybenzamine	4	489 ± 35†	918 ± 38‡	35 ± 7†

The results are means ± SEM (min), $*p<0.05$ by one-way ANOVA for Non RD, RD Control and Bromocriptine with comparisons made to Non RD Control by the Scheffe test. N—number of animals.

$\dagger p<0.05$ and $\ddagger p<0.0005$ by one-way ANOVA for Non RD, RD Control and Phenoxybenzamine with comparisons made to Non RD Control by the Scheffe test.

TABLE 3

EFFECTS OF BROMOCRIPTINE (5 mg/kg, IP) AND PHENOXYBENZAMINE (10 mg/kg, IP) ON WAKEFULNESS, SWS AND REM SLEEP IN RATS DURING 24–36 HR AFTER REM SLEEP DEPRIVATION. DRUGS WERE GIVEN DURING REM SLEEP DEPRIVATION

	N	Wakefulnes	SWS	REM Sleep
Non-RD Control	5	202 ± 20	435 ± 16	80 ± 6
RD Control	8	232 ± 14	425 ± 13	63 ± 5
Bromocriptine	8	225 ± 18	434 ± 10	62 ± 10
Phenoxybenzamine	4	168 ± 16	472 ± 23	81 ± 7

The results are means ± SEM (min). N—number of animals.

RESULTS

Effects of bromocriptine administration on wakefulness, SWS or REM sleep during the first 24 hours after RD (Table 2) as well as during the next 24–36 hour period (Table 3) did not differ from findings in non-RD control group. In particular, there was no rebound of REM sleep during the entire 36 hour recording period (Fig. 1). Moreover, during the first 6 hours after RD, REM sleep was reduced in comparison to REM sleep of the non-RD group for the same time period (Fig. 1). This reduction may have been due to a carry-over effect of repeated administration of bromocriptine beyond the six hours reported previously as a duration of action of a single 5 mg/kg dose of bromocriptine on REM sleep [9].

Administration of phenoxybenzamine in Experiment 1 (i.e., 18 hours prior to termination of RD) resulted in decreased REM sleep and wakefulness and increased SWS during the first 24 hours after RD (Table 2) but had no effect on sleep during the next 24–36 hour period (Table 3). Accordingly, there was no REM sleep rebound following RD (Fig. 2). However, during the first 12 hours after RD, REM sleep was significantly reduced when compared to REM sleep of the non-RD control group.

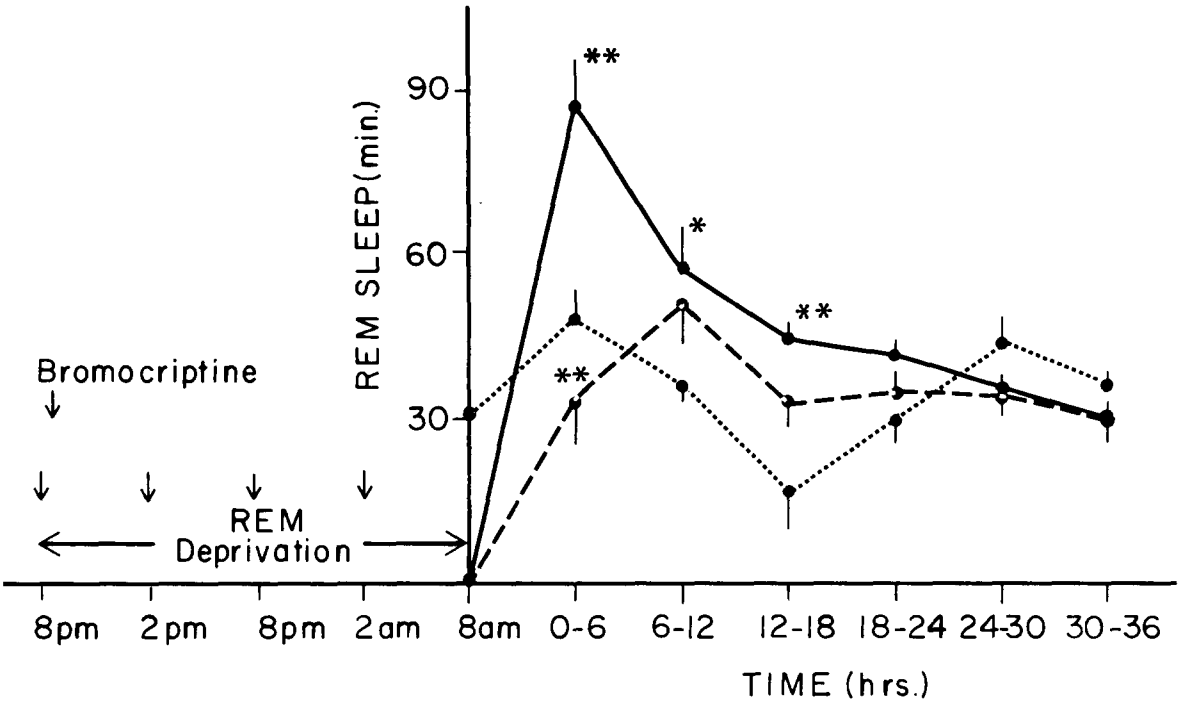

FIG. 1. Effect of bromocriptine on REM sleep rebound in rats. Bromocriptine was administered to rats during REM sleep deprivation. Non-RD control (· · ·), RD control (——), bromocriptine (---). $*p<0.05$, $**p<0.01$ by two-way ANOVA with comparisons made to the non-RD control by Newman-Keul's test.

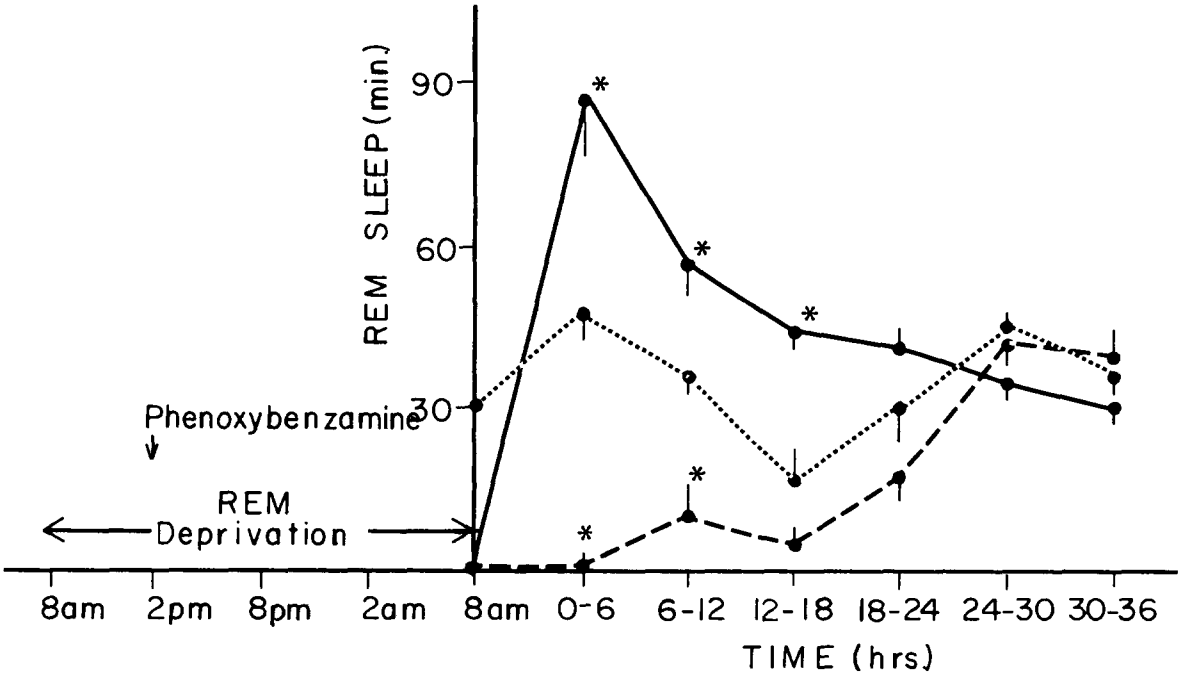

FIG. 2. Effect of phenoxybenzamine on REM sleep rebound in rats. Phenoxybenzamine was administered to rats during REM sleep deprivation. Non-RD control (· · ·), RD control (——), phenoxybenzamine (---). $*p<0.01$ by two-way ANOVA with comparisons made to the non-RD control by Newman Keul's Test.

TABLE 4

ADMINISTRATION OF PHENOXYBENZAMINE (10 mg/kg, IP) TO RATS PRIOR TO 48 HR OF REM SLEEP DEPRIVATION. EFFECTS ON WAKEFULNESS, SWS AND REM SLEEP DURING 0–30 HR AFTER REM SLEEP DEPRIVATION

	N	Wakefulness	SWS	REM Sleep
Non-RD Control	5	661 ± 31	974 ± 27	163 ± 8
RD-Control	5	658 ± 54	853 ± 50	289 ± 17*
Phenoxybenzamine	5	778 ± 90	826 ± 60	199 ± 39

The results are expressed as means ± SEM (min) *$p<0.05$ by Steel's non parametric test with comparison to Non-RD Control. N—number of animals.

TABLE 5

ADMINISTRATION OF PHENOXYBENZAMINE (10 mg/kg, IP) TO RATS PRIOR TO 48 HR OF REM SLEEP DEPRIVATION. EFFECTS ON WAKEFULNESS, SWS AND REM SLEEP DURING 30–60 HR AFTER REM SLEEP DEPRIVATION

	N	Wakefulness	SWS	REM Sleep
Non-RD Control	5	745 ± 20	899 ± 20	154 ± 3
RD Control	5	750 ± 54	878 ± 47	167 ± 24
Phenoxybenzamine	5	758 ± 33	894 ± 34	146 ± 7

The results are expressed as mean ± SEM (min). N—number of animals.

In the Experiment 2, phenoxybenzamine was administered at the beginning of the 48 hours of RD and produced an effect on sleep only during the first 30 hours following RD (Table 4). During this time period there was no REM sleep rebound since the amount of REM sleep in the phenoxybenzamine treated animals was not different from the REM sleep of the non-RD control rats. Also, during 30–60 hour periods there was no difference in the amount of REM sleep between RD control, non-RD control and phenoxybenzamine treated groups (Table 5). Comparable results obtained for REM sleep in all three animal groups during the 30–60 hour period after RD further indicated that a normalization of the sleep-waking pattern in the RD control and phenoxybenzamine treated rats occurred. This suggests that the lack of REM sleep rebound following the administration of phenoxybenzamine during RD, results from the drug's ability to fulfill REM sleep need.

Administration of phenoxybenzamine in Experiment 2 had no effect in wakefulness or SWS (Tables 4 and 5).

DISCUSSION

The results show that when the drug action of phenoxybenzamine or bromocriptine occurs during the RD period, the agents were able to prevent subsequent appearance of REM sleep rebound. In Experiment 1, in order to cover the entire 24 hour RD period, bromocriptine was administered four times in 6 hour intervals. Also, although the action of a single injection of phenoxybenzamine in Experiment 1 did not involve the initial 6 hours of 24 hour RD

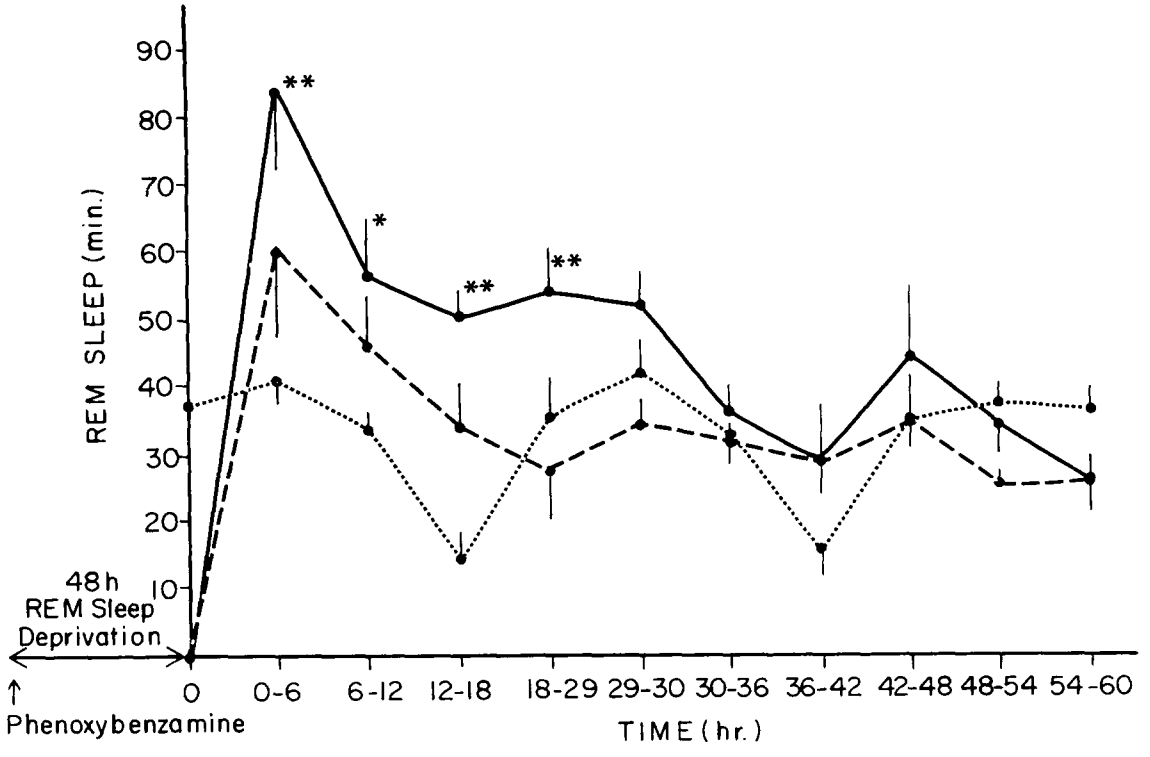

FIG. 3. Effect of phenoxybenzamine on REM sleep rebound in rats. Phenoxybenzamine was administered to rats prior to 48 hours of REM sleep deprivation. Non-RD control (· · ·), RD control (——), phenoxybenzamine (---). *$p<0.05$, **$p<0.01$ by two-way ANOVA with comparisons made to the non-RD control by Newman-Keuls's test.

period, it completely eliminated REM sleep rebound and even reduced REM sleep during the first 12 hours following RD to levels below that seen in normal rats. Since these effects exceeded 18 hours of RD period indicating that phenoxybenzamine action lasted for 30 hours, we extended RD period for another 24 hours in Experiment 2 and administered the drug to rats before the onset of this 48 hour period. Statistical analysis has shown that the amount of REM sleep in phenoxybenzamine treated animals did not differ from REM sleep in non-RD group of rats during each of the 6 hour intervals of the entire post-RD 60 hour recording period (Fig. 3).

If REM sleep rebound is a result of the increased pressure of suppressed REM sleep or of an increased need for REM sleep, then compounds which abolish REM sleep rebound may also act by fulfilling the need for REM sleep or by substituting pharmacologically for it. Stern and Morgane have proposed that a drug may reduce REM sleep by either disrupting the mechanism of REM sleep or by fulfilling REM sleep "need" [11]. Upon drug withdrawal, those agents which disrupt the mechanism show a subsequent REM sleep rebound. In the latter case no REM sleep rebound occurs after drug's suppression of REM sleep and the authors interpreted this as the drug's fulfillment of a possible neurochemical function of REM sleep.

The function of REM sleep is unknown as is the means by which either phenoxybenzamine or bromocriptine may fulfill REM sleep "need." Although it may be premature to speculate about drug fulfillment of the neurochemical function of REM sleep, a common neurochemical denominator of phenoxybenzamine and bromocriptine could be reduced transmission in the central noradrenergic system. Phenoxybenzamine is a potent α-adrenergic receptor blocker [8] and its action involves predominantly post-synaptic α_1 receptors [4]. In our previous work we measured in rat brains 3 methoxy-4-hydroxyphenylethylen-glycol sulfate (MOPEG-SO_4), a final product of norepinephrine metabolism, after the administration of the same dose of phenoxybenzamine (10 mg/kg) to rats and found concentration of MOPEG-SO_4 increased, which indicated effective drug action on central α-adrenoreceptors [10].

Bromocriptine, primarily recognized as a dopamine receptor stimulant [3] has been reported to possess some action on central noradrenergic system. A study in humans indicates a decrease in norepinephrine release following bromocriptine administration [13] while findings in rats show that bromocriptine may also act as an α-adrenergic blocking agent [12]. The effect in humans could be explained as an action of a low dose of bromocriptine (1–2 mg/kg) on dopamine receptors located on presynaptic noradrenergic nerve terminals. Stimulation of these receptors by bromocriptine has been suggested to inhibit norepinephrine release. The effect of a high dose of bromocriptine in rats (10 mg/kg) which produced an increase in MOPEG-SO_4 in the brain could be a result of an α-adrenoreceptors blockade [12]. Similar effect on central α-adrenoreceptors in rats has been reported for several other ergot compounds [2,12].

Previously, we have postulated that reduced noradrenergic transmission in the brain may abolish REM sleep rebound and our findings were consistent with that postulate [10]. Present results also support that hypothesis. Furthermore, present data show that administration of phenoxybenzamine or bromocriptine to rats immediately before or during REM sleep deprivation can prevent generation of REM sleep pressure by fulfilling the need for REM sleep since both treatments resulted in elimination of the subsequent REM sleep rebound.

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