Pharmacology Biochemistry & Behavior, Vol. 13, pp. 51–55. Printed in the U.S.A.

Elimination of REM Sleep Rebound in Rats by α-Adrenoreceptor Blockers, Phentolamine and Phenoxybenzamine

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Received 8 March 1980

RADULOVACKI, M., W. J. WOJCIK, C. FORNAL AND R. MILETICH. *Elimination of REM sleep rebound in rats by α-adrenoreceptor blockers, phentolamine and phenoxybenzamine.* PHARMAC. BIOCHEM. BEHAV. 13(1) 51–55, 1980.—Two α-adrenoreceptor blocking agents, phentolamine (5 mg/kg, IP) and phenoxybenzamine (10 mg/kg IP) were administered to rats deprived of rapid eye movement (REM) sleep for 24 hours to test the hypothesis that reduced noradrenergic transmission may abolish REM sleep rebound. The hypothesis was based on results from our previous studies which showed that administration to rats of diethyldithiocarbamate (DDC), a dopamine beta hydroxylase inhibitor, decreased the concentration of brain norepinephrine and reduced REM sleep permanently without the subsequent appearance of REM sleep rebound. Present results show that administration of both α-adrenoreceptor blockers abolished REM sleep rebound. At the time of maximum reduction of REM sleep, the concentration of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate ($MOPEGSO_4$), a final product of norepinephrine metabolism in the brain, was found increased in the whole brains of rats suggesting that the selected doses of the drugs were sufficient to produce effective central α-adrenergic receptor blockade. These data indicate that the action of both α-adrenoreceptor blocking agents in noradrenergic system was paralleled by the permanent loss of REM sleep and support the hypothesis implicating reduced noradrenergic transmission in elimination of REM sleep rebound.

Phentolamine Phenoxybenzamine Elimination of REM sleep rebound

THE aim of this work was to test the hypothesis that reduced noradrenergic transmission may abolish rapid eye movement (REM) sleep without producing subsequent REM sleep rebound. The hypothesis was based on results from our previous studies which showed that administration to rats of diethyldithiocarbamate (DDC), a dopamine (DA) beta hydroxylase inhibitor [4], decreased the concentration of norepinephrine (NE) in specific brain structures [25] and reduced REM sleep permanently without the subsequent appearance of REM sleep rebound [11]. However, since the inhibition of DA beta hydroxylase resulted in a decrease of brain NE content, it was not certain whether this reduction actually affected noradrenergic transmission.

In attempting to reduce noradrenergic transmission by a different approach and to examine its effect on REM sleep rebound we used α-adrenoreceptor blockers in a situation in which REM "pressure" is increased by a selective REM deprivation method [7,16] and as a result REM sleep appears in a greater amount. We administered two α-adrenoreceptor blockers, phentolamine [18] and phenoxybenzamine [19] to rats selectively deprived of REM sleep. Our results show that administration of both agents abolished REM sleep rebound and, in the case of phenoxybenzamine, REM sleep was reduced to levels below that seen in normal rats.

METHOD

Implantation of Electrodes, REM Sleep Deprivation and Polygraphic Recording

Adult male Sprague-Dawley rats (400–500 g) were implanted 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" method where animals are placed on platforms surrounded by water [7]. The method is based on the phenomenon that during REM sleep there is a loss of muscle tone which causes the animals to fall off the platforms. The animals awake at the onset of each REM episode and thus are deprived of REM sleep. To standardize the degree of REM deprivation (RD) rats were placed on circular platforms whose surface area corresponded to their body weight [6,16]. A surface area to animal weight ratio of 14 cm^2/100 g was used. After 24 hr of RD, animals were divided in three groups. The animals in the first group received phentolamine hydrochloride (5 mg/kg, IP), those in the second group received phenoxybenzamine hydrochloride (10 mg/kg, IP) and the rats in the third, control, group received the drug vehicle, propylene glycol (0.5 ml/kg, IP). After the drugs were administered, EEG and EMG were continously monitored for

TABLE 1

THE EFFECT OF PHENTOLAMINE AND PHENOXYBENZAMINE ADMINISTRATION ON REM SLEEP AFTER 24 HOURS OF REM DEPRIVATION IN RATS

Hours after REM deprivation	REM deprived control	Phentolamine 5 mg/kg REM sleep mean±SE (min)	Difference from control (min)	Significance*	Phenoxybenzamine 10 mg/kg REM sleep mean±SE (min)	Difference from control (min)	Significance*
0– 6	47 ± 3 (6)	25 ± 5 (7)	−22	$p<0.005$	5 ± 2 (6)	− 42	$p<0.0005$
6–12	49 ± 3 (6)	43 ± 3 (7)	− 6	NS	15 ± 5 (6)	− 34	$p<0.0005$
12–18	37 ± 4 (6)	40 ± 2 (7)	+ 3	NS	21 ± 5 (6)	− 16	$p<0.05$
18–24	28 ± 3 (6)	40 ± 5 (7)	+12	NS	20 ± 7 (6)	− 8	NS
24–30	21 ± 4 (6)	24 ± 4 (7)	+ 3	NS	23 ± 4 (6)	+ 2	NS
30–36	26 ± 2 (6)	25 ± 2 (7)	− 1	NS	24 ± 5 (6)	− 2	NS
36–42	22 ± 4 (4)	35 ± 6 (5)	+13	NS	26 ± 7 (6)	+ 4	NS
42–48	29 ± 6 (4)	33 ± 4 (5)	+ 4	NS	30 ± 6 (4)	+ 1	NS
0–24	161 ± 3 (6)	149 ± 8 (7)	−12	NS	61 ± 11 (6)	−100	$p<0.005$
24–48	99 ± 12 (4)	121 ± 12 (5)	+22	NS	99 ± 22 (4)	0	NS
0–48	257 ± 13 (4)	278 ± 14 (5)	+21	NS	147 ± 33 (4)	−110	$p<0.005$

Numbers in parentheses indicate the number of animals in the experiment.
*Statistics by one-way ANOVA with multiple comparisons performed by Scheffe Test. NS=no significance.

TABLE 2

THE EFFECTS OF PHENTOLAMINE AND PHENOXYBENZAMINE ON WAKEFULNESS AFTER 24 HOURS OF REM DEPRIVATION IN RATS

Hours after REM deprivation	REM deprived control	Phentolamine 5 mg/kg	Phenoxybenzamine 10 mg/kg
0– 6	136 ± 13 (6)	180 ± 12 (7)	163 ± 17 (6)
6–12	100 ± 12 (6)	132 ± 11 (7)	115 ± 12 (6)
12–18	101 ± 6 (6)	115 ± 13 (7)	106 ± 34 (6)
18–24	103 ± 5 (6)	104 ± 11 (7)	103 ± 14 (6)
0–24	440 ± 21 (6)	531 ± 19 (7)	487 ± 58 (6)
24–48	506 ± 53 (4)	513 ± 24 (5)	442 ± 42 (4)
0–48	949 ± 82 (4)	1,033 ± 40 (5)	919 ± 119 (4)

The results are means ± SE (min). Numbers in parentheses indicate the number of animals in the experiment.

TABLE 3

THE EFFECTS OF PHENTOLAMINE AND PHENOXYBENZAMINE ON SLOW WAVE SLEEP AFTER 24 HOURS OF REM DEPRIVATION IN RATS

Hours after REM deprivation	REM deprived control	Phentolamine 5 mg/kg	Phenoxybenzamine 10 mg/kg
0– 6	177 ± 12 (6)	155 ± 9 (7)	193 ± 19 (6)
6–12	211 ± 10 (6)	184 ± 9 (7)	230 ± 12 (6)
12–18	222 ± 3 (6)	205 ± 11 (7)	232 ± 30 (6)
18–24	230 ± 5 (6)	216 ± 7 (7)	237 ± 13 (6)
0–24	838 ± 21 (6)	760 ± 15 (7)	893 ± 55 (6)
24–48	835 ± 42 (4)	805 ± 15 (5)	900 ± 33 (4)
0–48	1,673 ± 71 (4)	1,568 ± 30 (5)	1,814 ± 95 (4)

The results are means ± SE (min). Numbers in parentheses indicate the number of animals in the experiment.

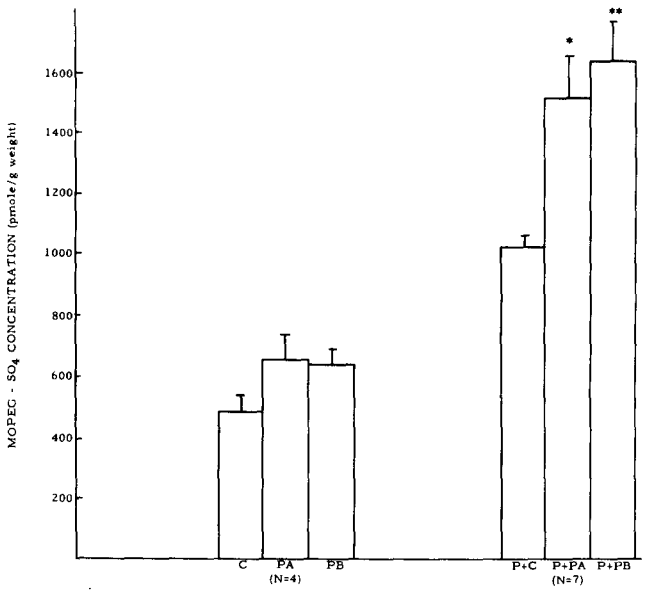

FIG. 1. The effects of phentolamine (5 mg/kg IP) and phenoxybenzamine (10 mg/kg IP) with or without probenecid (20 mg/kg IP) on the concentration of MOPEG-SO_4 in the whole brains of rats deprived of REM sleep for 24 hrs. The results are means ± SE, C=Control, PA=Phentolamine, PB=Phenoxybenzamine, P=Probenecid, N=Number of animals. *$p<0.05$ and **$p<0.01$ by Steel's Nonparametric Test.

48 hours. All records were analyzed for wakefulness, slow-wave sleep (SWS) and REM sleep. Evaluation of the polygraphic records was made using standard techniques where each epoch of record was determined to be either wakefulness, SWS or REM. The epochs were 50 sec long and the speed of the paper drive was 100 sec/page of paper. The data obtained at 6 hour intervals were submitted to statistical analysis. The statistical test used to determine significant differences among groups was the one way analysis of variance (ANOVA) for each time period and for each state. For those with significant difference among groups, the Scheffe' multiple comparison method was applied.

Determination of MOPEG SO_4

Adult male Sprague-Dawley rats (300–350 g) were REM deprived for 24 hr (8 a.m.–8 a.m.). At 8 a.m. following REM deprivation animals received phentolamine (5 mg/kg, IP), phenoxybenzamine (10 mg/kg, IP) or the drug vehicle propylene glycol (0.5 ml/kg, IP). At 9 a.m. some of the animals of the three groups received probenecid (200 mg/kg, IP). At 11 a.m. all animals were decapitated and their brains were removed, weighed and assayed the same day for 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MOPEG-SO_4), a final product of NE metabolism. The assay procedure used was that of Meek and Neff [15] with minor modification by McMillen and Shore [14].

RESULTS

Our previous findings [22] show that the method used for REM sleep deprivation of rats in this study produces a selective rebound only of REM sleep and not of SWS in RD rats. Also, the obtained REM sleep rebound was the greatest during the first 5 hours of the EEG recording period after RD.

Results of the effect of phentolamine on REM sleep in RD rats are shown in Table 1. The table shows that a dose of 5 mg/kg phentolamine abolished REM sleep rebound during the first 6 hr period. At the end of the 24 hr period after RD the difference between control and phentolamine groups was not statistically significant. Also, there was no difference at the end of 48 hr EEG recording period after RD between control and experimental groups.

Administration of the 10 mg/kg dose of phenoxybenzamine (Table 1) abolished REM sleep rebound and significantly reduced the amount of REM sleep that would have normally been seen without RD for 18 hours following drug administration. This REM sleep reducing effect gradually diminished during the first 24 hr after RD. During the second 24 hr period after RD total REM sleep in the experimental group was identical to REM sleep in the control group, indicating normalization of the sleep-waking pattern. Since the amount of REM sleep lost during the first 24 hr period after RD was not regained during the second 24 hr period, this finding suggests that administration of phenoxybenzamine eliminated REM sleep during that time period.

Tables 2 and 3 show the effects of phentolamine and phenoxybenzamine on wakefulness or SWS in RD rats. The results indicate that neither wakefulness nor SWS were significantly affected by either drug treatment. According to these findings it appears that noradrenergic transmission through α-adrenoreceptors is not an integral part in the mechanism of wakefulness or SWS.

Results of brain MOPEG-SO_4 determination are shown in Fig. 1. Although the concentration of MOPEG-SO_4 following administration of phentolamine or phenoxybenzamine alone was not statistically different from control, the combination of either drug with probenecid post-treatment resulted in a significant increase in MOPEG-SO_4 concentration. These results suggest that effective α-adrenoreceptor blockade was achieved by administration of phentolamine or phenoxybenzamine.

DISCUSSION

Although there are no reports on the effects of phenoxybenzamine or phentolamine on REM sleep rebound, there have been studies on the effects of α-adrenoreceptor blockers on REM sleep. The present results with phenoxybenzamine are in agreement with findings of Matsumoto and Watanabe [13] who showed a reduction of REM sleep in cats with 15 mg/kg phenoxybenzamine. However, with oral administration of 40 mg/kg phenoxybenzamine REM sleep was increased in rats 8 hours following drug's administration [5]. Interestingly enough, the same authors reported little or no effect on REM sleep using oral administration of 2, 5, 10, 20 or 80 mg/kg phenoxybenzamine.

Our results with phentolamine are in accordance with findings of Makela and Putkonen in rats [12] which showed that administration of phentolamine (10 mg/kg) reduced REM sleep. Since in that study the polygraphic recordings were only for 12 hr it is not possible to draw any conclusions on drug's effect on REM sleep rebound.

In order to demonstrate that the effects with phentolamine and phenoxybenzamine on REM sleep involved central noradrenergic mechanism, it was necessary for us to determine, by a chemical method, that the dosages selected in the present study were sufficient to block central

α-adrenoreceptors. Results obtained with probenecid show that MOPEG-SO_4, the end product of NE metabolism in the brain, significantly increased in the whole rat brains following administration of both drugs (Fig. 1). This finding indicates that an effective central α-adrenoreceptor blockade was achieved.

It is of interest that in an identical experimental design of our previous study [22], administration of bromocriptine, a DA receptor stimulant [20] also abolished REM sleep rebound. According to Vigouret *et al.* [24] and Burki *et al.* [2] bromocriptine may act as an α-adrenergic blocking agent in rats, while findings of Ziegler *et al.* [27] in humans indicate a decrease in NE release following bromocriptine's administration. Whether the first or the latter process is involved, it is conceivable that bromocriptine's effect on REM sleep was accomplished via a noradrenergic mechanism while the agonist action of bromocriptine on post-synaptic DA receptors has been associated with wakefulness. This double action of bromocriptine resulted in elimination of REM sleep rebound and in a reciprocal increase in wakefulness, whereas present administration of phentolamine and phenoxybenzamine was followed only by the lack of REM sleep rebound. In both situations, common behavioral and functional denominators were the reduction of REM sleep and reduced transmission in noradrenergic system.

Present data are in line with our previous work which showed that a decreased NE concentration in specific brain structures obtained after administration of DDC to rats was paralleled by the lack of REM sleep rebound [11]. Norepinephrine's role has been implicated in REM sleep [8,26] and there is a series of reports showing that agents which decrease NE at the synapse reduce REM sleep. One of the agents, α-methylparatyrosine which decreased both DA and NE concentrations in the whole brains of rats reduced significantly REM sleep [9]. Also, administration of presynaptic α-adrenoreceptor agonists, which inhibit NE release, clonidine and xylazine to cats [21] and clonidine to rats [10] and humans [13] reduced REM sleep. In accordance, administration of fusaric acid, a DA beta oxidase inhibitor [17], reduced REM sleep in cats [23]. However, REM sleep rebound was reported with moderate but not with large doses of the drug.

In conclusion, our results are consistent with the hypothesis that reduced noradrenergic transmission may abolish REM sleep rebound. Administration of phentolamine and phenoxybenzamine clearly abolished REM sleep rebound for 6 and 18 hours respectively. Also, at the time of maximum reduction of REM sleep the concentration of MOPEG-SO_4 in the brain increased indicating effective drug action on central α-adrenoreceptors.

ACKNOWLEDGEMENTS

This research was supported by ONR contract N00014-79-C-0420. Ciba-Geigy supplied phentolamine (Regitin) and Smith, Kline and French supplied phenoxybenzamine for these experiments.

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