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Central Responses to Cholinergic Drugs of REM Sleep Deprived Rats[1]

RICARDO SANTOS

Setor de Psiconeurobiologia, Instituto de Biologia
Universidade do Estado do Rio de Janeiro, Av. 28 de Setembro, 87 Fundos
20551, Rio de Janeiro, Brasil

AND

E. A. CARLINI

Departamento de Psicobiologia, Escola Paulista de Medicina
São Paulo, São Paulo, Brasil

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SANTOS, R. AND E. A. CARLINI. *Central responses to cholinergic drugs of REM sleep deprived rats.* PHARMACOL BIOCHEM BEHAV **29**(2) 217–221, 1988.—The present work studied the effects of REM sleep deprivation on the responses to cholinomimetic drugs in rats. Cataleptic behavior induced by pilocarpine, oxotremorine and eserine was not modified by previous REM sleep deprivation. On the other hand, the intensity of oxotremorine- and eserine-induced tremors, but not that of pilocarpine, was clearly augmented in the REMd rats and latency to the first tremor was shorter. REM sleep deprivation also potentiated the convulsions induced by nicotine. Two hypothetical mechanisms through which REM sleep deprivation could lead to the described hyperresponsiveness to cholinomimetic drugs are discussed.

REM sleep deprivation Cholinomimetic drugs Catalepsy Tremor Nicotine-induced convulsion Supersensitivity

THE effects of many drugs known to interfere with central neurotransmitter systems are different in rats deprived of rapid eye movement sleep (REMd rats), when compared to control (non-deprived) animals (for review, see [3]). For instance, REMd rats exhibit exaggerated responses to dopaminergic agents such as apomorphine, bromocriptine or piribedil [15,21]; the behavioral effects of serotoninergic agents are modified by previous REM sleep deprivation [15,18]. It is not clear whether such changes are due to presynaptic events or to modifications in the sensitivity of postsynaptic receptors [4, 22, 27].

The behavioral responses of REMd rats when challenged with cholinergic drugs have not been assessed. Alterations in the effects of such drugs could occur, at least theoretically, as a consequence of an altered dopaminergic influence on the cholinergic system or as a result of a direct influence of REM sleep deprivation on the dynamics of central cholinergic neurons.

Changes in central muscarinic receptor sensitivity, under various experimental conditions, have been studied using neurochemical techniques [12,20] or by measuring the responses induced by drugs, on behavioral parameters, such as changes in motor activity [5,14], induction of catalepsy [1, 9, 26] and induction of tremor [11,17].

In the present study the effects of four cholinergic drugs (pilocarpine, oxotremorine, eserine and nicotine) in REMd and control rats were compared. To evaluate the central cholinergic effects we selected catalepsy and tremor induced by eserine, pilocarpine and oxotremorine, and convulsion induced by nicotine.

METHOD

Subjects and REM Sleep Deprivation

Three to four-month-old male Wistar rats were used. REM sleep deprivation was achieved by placing the animals on a round platform 6 cm in diameter within a container filled with water at a level 1 cm below the top of the platform. Food was available ad lib. After 3 days the animals were removed from the platform, injected with one of the test drugs and the behavioral parameters observed. Control rats were maintained for 3 days in individual home wire cages measuring 15×20×30 cm. An additional control group ("wet controls") was maintained for 3 days in the containers but the

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platform was 14 cm in diameter. These procedures have been routinely used in our laboratories [23].

Drugs

Pilocarpine hydrochloride (Merck, Sharp and Dohme Lab), oxotremorine sesquifumarate (Aldrich Chemical Company), eserine sulphate (Sigma Chemical Co.) and nicotine (British Drug House Ltd.) were dissolved in distilled water just before use and injected in a volume of 0.1 ml/100 g body weight intraperitoneally. For control of solvent effect distilled water was injected in same volume.

Experiment 1—Cataleptic Effect Induced by Cholinergic Drugs

Eighty-four rats were deprived of REM sleep for 3 days and 84 others were maintained individually in their home cages for the same period of time. Five to 10 min after the end of the deprivation, REMd and control rats were injected with 5, 10 and 20 (12 REMd and 12 controls at each dose) mg/kg of pilocarpine; 0.25, 0.5 and 1.0 (8 REMd and 8 controls) mg/kg of oxotremorine; and 0.25, 0.5 and 1.0 (8 REMd and 8 controls) mg/kg of eserine. After the injections, the rats were individually housed in small wooden cages (10×10×20 cm). Ten min later they were removed from the cages and catalepsy was measured from 10 to 15, 35–40 and 60–65 minutes. At the end of each measurement they were reintroduced into the small wooden cages.

To measure catalepsy, the anterior forepaws of a rat were gently placed on a glass rod suspended 10 cm from the floor. Each animal was placed on the glass rod a maximum of three times during each 5 min test period and the total time it remained with the forepaws on the road was scored with a stopwatch.

The cataleptic effect of each dose of the drugs was compared between control and REMd groups using the Student's *t*-test.

Experiment 2—Tremor Induced by Cholinergic Drugs

Sixty-eight control rats and 69 REMd rats were employed. The animals were injected with 10 (7 controls and 7 REMd), 20 (6 and 6), 40 (6 and 6) and 80 (2 and 2) mg/kg of pilocarpine; 0.25 (9 controls and 10 REMd), 0.5 (8 and 8) and 1.0 (8 and 8) mg/kg of oxotremorine; and 0.5 (6 and 6), 0.75 (8 and 8) and 1.0 (8 and 8) mg/kg of eserine.

Immediately after the injection of the test drug, each rat was individually placed into a wire cage measuring 15×20×30 cm for observation. Latency for the appearance of the first tremor was scored as well as the intensity of tremor. The intensity was measured at 5–10, 25–30 and 55–60 min after drug administration, using a scale adapted from Weinstock *et al.* [24]: Grade 0—absence of tremor; grade 1—one to five head tremors, each with duration smaller than one min; grade 2—six or more head tremors with duration less than one min; grade 3—one to five head tremors with duration equal or larger than one min; grade 4—head tremor(s) during more than 1 min and one to five body tremors, each with duration less than one min; grade 5—head tremor (s) during more than 1 min and 6 or more body tremors during less than one min each; grade 6—head and body tremors during more than 1 min.

In a complementary experiment, 10 control rats and 10 "wet control" rats were treated with 0.5 mg/kg of oxotremorine and scored for latency and intensity of tremor.

Comparisons between control and REMd groups for each dose of the drug used or between control and "wet control" groups were made using the Student's *t*-test (for latency of tremor) and the Mann Whitney U-test (for intensity of tremor).

TABLE 1

INFLUENCE OF PREVIOUS REM SLEEP DEPRIVATION (REMd) ON THE CATALEPSY INDUCED BY CHOLINERGIC DRUGS IN RATS

Drug	Dose (mg/kg)	Total score (*) of catalepsy (sec±S.E.) of: Control Rats	REMd Rats
Pilocarpine	5	25 ± 15	7 ± 1
	10	20 ± 8	31 ± 10
	20	151 ± 38	127 ± 30
Oxotremorine	0.25	20 ± 5	33 ± 16
	0.5	87 ± 33	178 ± 33
	1.0	169 ± 23	124 ± 31
Eserine	0.25	5 ± 1	11 ± 3
	0.5	28 ± 9	32 ± 6
	1.0	65 ± 25	46 ± 15

*Total score represents the summation of the scores obtained during the period of 10–15, 35–40 and 60–65 min.

Experiment 3—Convulsions Induced by Nicotine

Thirty-seven control rats and 38 REMd rats were injected immediately after the end of the deprivation with 1.0 (4 controls and 4 REMd), 1.5 (8 and 9), 1.75 (12 and 12) and 2.0 (13 and 13) mg/kg of nicotine. After the injection, the animals were introduced in the wire cages and the occurrence of convulsion was scored by recording the presence or absence of any convulsive episode (clonic and/or tonic) elicited within 60 min after the IP injection of nicotine. Statistical analyses were performed using the Fisher test.

RESULTS

Experiment 1

Table 1 shows that REMd and control rats treated with several doses of three cholinergic drugs did not present differences between the groups when comparisons were done using total scores of catalepsy. Also, no differences were noted when the scores for the different time blocks (periods of 10–15, 35–40 and 60–65 min) of catalepsy were used for comparisons between groups.

Experiment 2

Figure 1 shows that for the doses of 0.25 and 0.5 mg/kg of oxotremorine and 0.75 mg/kg of eserine, REMd rats had a significantly shorter latency for tremor. Pilocarpine at the doses used did not induce tremor in either group of animals. Data from groups injected with 0.5 mg/kg of eserine were not included in Fig. 1 because only one rat in each group displayed tremor.

As seen in Fig. 2, when treated with doses of 0.25, 0.5 and 1.0 mg/kg of oxotremorine and 0.75 mg/kg of eserine REMd rats showed a significantly larger intensity of tremor at the 5–10 min observation period. The same was also observed at

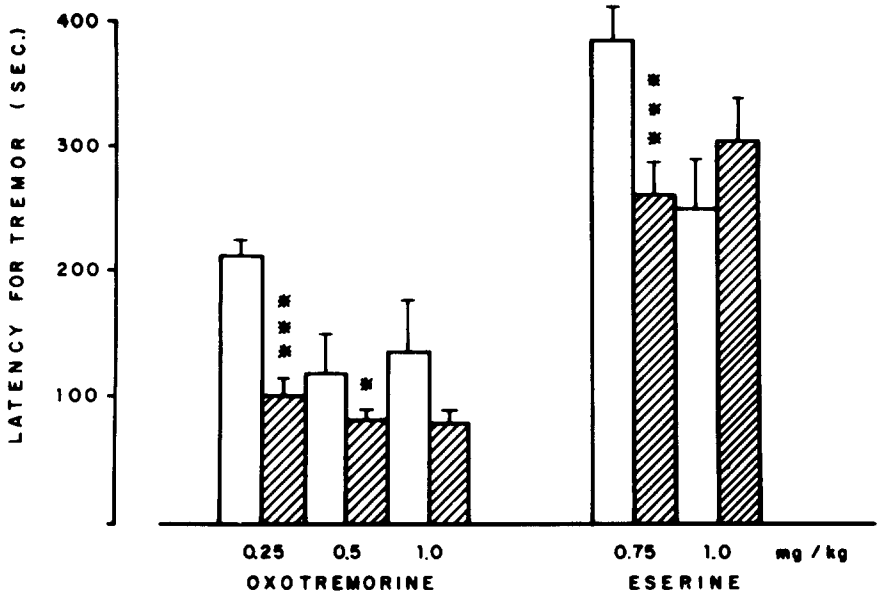

FIG. 1. Latency for the appearance of the first tremor in control (open columns) and REMd (hatched columns) rats after treatment with oxotremorine and eserine. The bars above the columns represent the standard errors of the means. Asterisks indicate statistically significant differences between groups (*$p \leq 0.05$; ***$p \leq 0.002$).

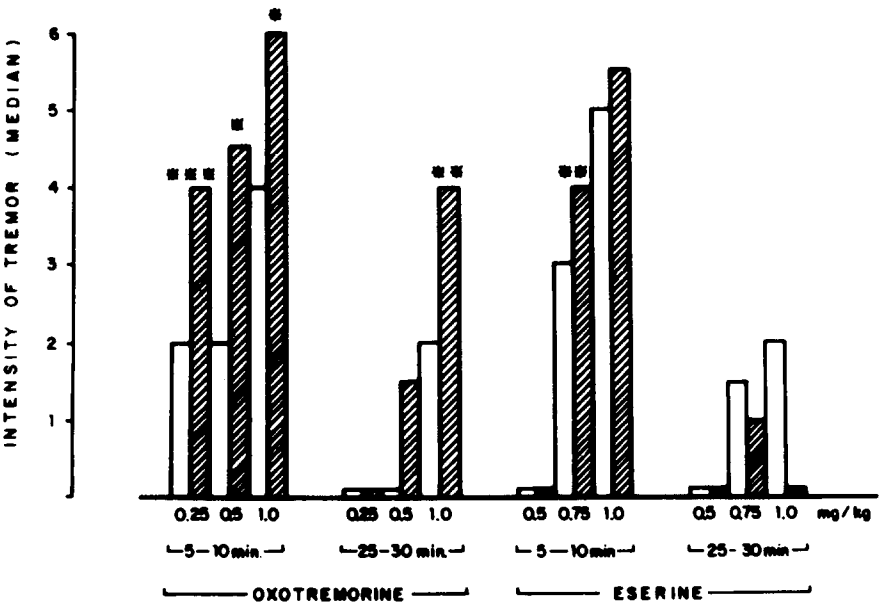

FIG. 2. Intensity of tremor scored in grades in control (open columns) and REMd (hatched columns) rats after treatment with oxotremorine and eserine (*$p \leq 0.05$; **$p \leq 0.01$ and ***$p \leq 0.002$).

25–30 min period for the dose of 1.0 mg/kg of oxotremorine.

On the other hand, the rats maintained for 3 days on the 14 cm platforms ("wet controls") did not differ from the control animals when both groups were treated with 0.5 mg/kg of oxotremorine (respectively, 136±12 and 143±16 sec for latency and degrees 2 and 2 for intensity of tremor). That is, the decrease of latency and the increase of intensity of tremor observed after REM sleep deprivation were not observed in stressed animals maintained on the large platforms.

Experiment 3

As can be seen in Fig. 3, a larger percentage of REMd rats displayed convulsions after treatment with doses of 1.75 and 2.0 mg/kg of nicotine, when compared to control animals.

DISCUSSION

Tremor is a behavioral parameter which has been used to evaluate the activity of central cholinergic systems and the

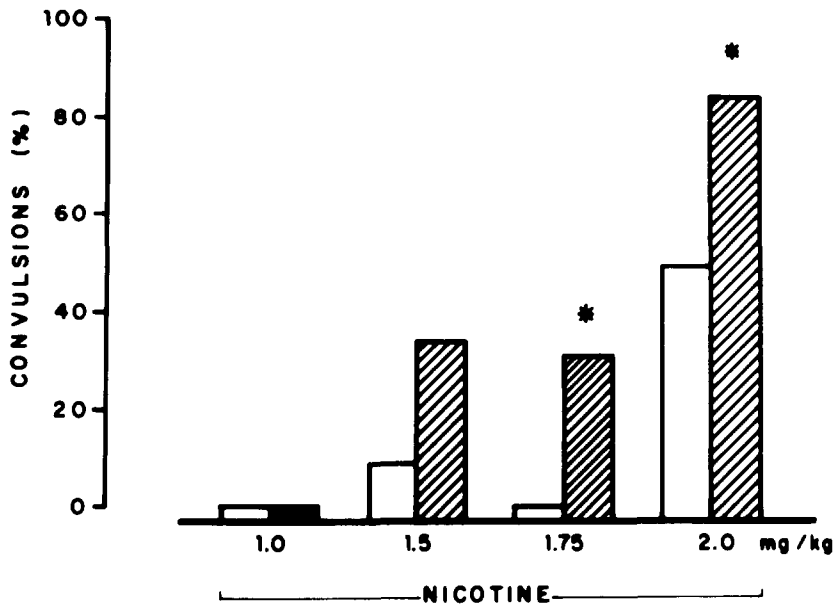

FIG. 3. Percentage of occurrence of convulsions in control (open columns) and REMd (hatched columns) rats after treatment with several doses of nicotine (*$p \leq 0.05$).

effects of cholinomimetic drugs such as oxotremorine [13,17]. Our results (experiment 2; Figs. 1 and 2) show that REMd rats displayed shorter latencies and greater amounts of tremor than control animals after both oxotremorine and eserine. There is no explanation for the failure of pilocarpine to induce tremor. This effect is most probably due to the REM sleep deprivation *per se*, as "wet control" rats did not present it. A similar potentiating effect by REM sleep deprivation was also obtained with the nicotine-induced convulsions (Fig. 3). On the other hand, REM sleep deprivation did not change the cataleptic response to cholinomimetic drugs (experiment 1; Table 1).

There are at least two possibilities to explain the potentiating effect of REM sleep deprivation on tremor and convulsions induced by these cholinomimetic agents. First, REM sleep deprivation could directly induce a change in the state of central cholinergic receptors. A state of neuronal supersensitivity to acetylcholine is produced by periodic brain stimulation (kindling) leading to electrical and behavioral seizures, probably by increasing number and/or sensitivity of postsynaptic cholinergic receptors [2]. In a similar fashion REM sleep deprivation by a yet unknown and direct mechanism could increase sensitivity of cholinergic receptors located in some central areas leading to the exaggerated tremor and convulsions induced by the cholinomimetics.

The second possibility lies in the described central dopaminergic hyperresponsiveness of REMd rats [15, 21, 23]. Dopaminergic neurons in some brain areas have an inhibitory influence on cholinergic neurons. Therefore, a heightened dopaminergic activity would lead a higher inhibition on cholinergic neurons resulting in a supersensitivity of their postsynaptic receptors. Such a state of supersensitivity has been obtained in rats by chronic treatment with anticholinergics [7, 20, 25], antidepressants [10], ethanol—which inhibits acetylcholine release [19]—and by the destruction of cholinergic neurons through medial septal lesion [25].

However, neither of the above mentioned possibilities explain the lack of influence of REM sleep deprivation on the cholinomimetic-induced catalepsy. One may wonder whether the changes in cholinergic function brought about by REM sleep deprivation could be specific to certain brain areas. For example, a similar phenomenon as that described for chronic ethanol administration could be occurring: ethanol by inhibiting acetylcholine release increases the number of cholinergic receptors in the hippocampus and cortex but not in the striatum [19]. It is known that the latter brain structure plays an important role in the cataleptic behavior [8,16]. It is pertinent to these findings that a 4-day period of REM sleep deprivation also does not change the striatal acetylcholine levels of rats [6].

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