Differential effects of scopolamine and chlorpromazine on REM and NREM sleep in normal male subjects*

The study compares the effects of scopolamine, methscopolamine, and chlorpromazine on the EEG and the EOG in sleeping subjects. Saline was used as a control. Scopolamine hydrobromide (0.006 mg. per kilogram) clearly retarded the onset of stage REM. No immediate rebound was seen in the analysis of the sleep pattern for two hours after the first recording of this stage. The total amount of REM sleep during the full period of recording was therefore diminished. The decrease in REM resulted in an increased amount of stages I and II. No significant changes were observed in stages III or IV or in the periods of wakefulness. A significant increase in body movements was noted. Methscopolamine bromide (0.0055 mg. per kilogram) did not produce any substantial modification in the pattern of sleep. Chlorpromazine hydrochloride (0.4 mg. per kilogram) produced an increase of stage III activity apparently at the expense of stage II sleep. The observed changes further support a role of cholinergic mechanisms in human REM sleep.

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The role of biogenic amines, particularly 5-hydroxytryptamine (5HT) and norepinephrine, in the mechanisms of sleep has been the subject of numerous studies.[7, 9, 13] Not as much attention has been paid to the possible participation of cholinergic mechanisms in the two major states of sleep—slow wave EEG (NREM) and rapid-eye movements with EEG fast waves (REM)—or in the transition from one into another. Animal studies, however, suggest that cholinergic mechanisms are involved in sleep. The muscarinic cholinergic antagonist atropine has been reported to suppress REM sleep in the cat.[7, 8] Other qualitative differences have been shown in this species depending upon the doses employed. On the other hand, direct injection of oxotremorine or carbachol into the brainstem reticular formation induces REM sleep in the cat.[1, 4]

To date no reports are available on the effects of muscarinic cholinergic antagonists on the human sleep cycle. Scopolamine, whose central nervous system effects are well recognized, was considered

Supported in part by grant MH-02653 from the United States Public Health Service.

*A preliminary report was presented at the American Society for Psychological Studies of Sleep meeting in Boston, March, 1969.

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an appropriate tool to elucidate the role of a cholinergic sleep mechanism in man, especially since it is often included in over-the-counter sleep-inducing preparations. Clinically, it has been noted that scopolamine induces "dreamless" sleep.[5] This could be due to a decrease in stage REM or to a decrease in dream recall. The quaternary nitrogen derivative of scopolamine, methscopolamine, which has negligible central nervous system effects but even greater peripheral effects,[16] was used to determine the relative importance of central and peripheral cholinergic blocking effects on sleep. The α-adrenergic antagonist chlorpromazine also produces sedation and EEG effects similar to those of scopolamine in the waking state. In this study it was used in clinically similar dosage to compare its effects with those of scopolamine.

Methods

Eight young healthy men, aged 21 to 29, participated in these experiments after a complete history and physical examination had been obtained. The presence of basal alpha rhythm in the occipital areas during a resting EEG record was required for the acceptance of each subject into the study. The participants were instructed to abstain from alcohol or any other pharmacologically active substance during the 24 hours prior to the beginning of the experiment. No coffee or tobacco was permitted after 7. P.M. The first night of sleep was used for habituation. The recordings were made in a sound-attenuated, temperature-controlled chamber. Electrodes for polygraphic recordings were placed in the usual positions (see below) but a continuous all-night recording was not made. The subjects did not know that this was the case, inasmuch as they were isolated in the recording chamber. The second night was used for the baseline recordings ("no medication" night). After the second night, a sequence of saline, methscopolamine Br (0.0055 mg. per kilogram), no medication, scopolamine HBr (0.006 mg. per kilogram), no medication, and chlorpromazine HCl (0.4 mg. per kilogram) injections followed in four of the individuals. In the other four subjects only two drugs (scopolamine and chlorpromazine or scopolamine and methscopolamine) were used. "No medication" nights were intercalated in all instances in order to avoid overlapping in drug effects.

Recording schedule. The subjects entered the sleep laboratory at 9:45 P.M. From 9:45 to 11:00 P.M. the polygraph electrodes were applied and an initial calibration of the polygraph was performed. A polygraph record was obtained with the subject keeping his eyes open, blinking, and also with his eyes closed. At approximately 11:00 P.M. the scheduled injection was given in accordance with the experimental design described above. In all cases the intramuscular route was used. Injections were given low in the deltoid muscle (providing opportunity for use of a tourniquet if necessary). Following injection, the lights in the sound-attenuated chamber were turned off and a continuous polygraph recording was begun. This lasted until 6:30 A.M. During all recording periods the room temperature was kept at a comfortable level, according to indivdual subject preferences. An intercom was available for two-way communication. Every effort was made to achieve a comfortable and "natural" environment for each individual, including the use of light or heavy pajamas, blankets, pillows, night lights, and the like, according to individual preference. All the drugs used in this study were prepared by the University Hospital pharmacy. The final volume to be injected was adjusted to 1.5 ml. in all cases.

Electrode placement. At the beginning of each recording night, silver disc scalp electrodes were placed at positions F_3, C_3, C_4, P_3 and O_1 as defined by the 10-20 International System.[6] Beckman self-adhering electrodes at F_{p1}, the outer canthi of both eyes and submentally, served as ground, electrooculogram (EOG) and electromyogram (EMG), respectively. Two

Telectrode self-adhering electrodes placed on the lobule of each ear and connected together served as reference. EEG, EMG, and EOG on an 8 channel Grass polygraph were recorded outside the sleeping chamber. A paper speed of 15 mm. per second and calibration of 50 μV per centimeter were used except for the EMG channel, which was calibrated at 10 μV per centimeter, and the EOG, which was calibrated at 100 μV per centimeter.

Scoring of sleep polygraph records. Modified criteria of Dement and Kleitman[3] and Williams and co-workers[17] were used. The entire sleep record of each night was divided into one-minute epochs, each epoch being scored as one of the following: wakefulness, stages I, II, III, IV, REM, or body movements. These features were scored as follows: Wakefulness (stage W), active awake, was characterized by low-voltage fast EEG patterns, EMG activity, and eye movements; quiet awake had a lower EMG and more than 50 per cent alpha rhythm (7.5 to 12.5 Hz.). Stage I was characterized by a low-voltage fast EEG pattern with less than 50 per cent alpha rhythm. Stage II contained at least two well-defined sleep spindles or two "K" complexes or one of each and no more than 20 per cent delta activity (frequencies from 0.5 to 4 Hz. and amplitudes of 30 or more μV). Stage III contained more than 20 per cent but less than 50 per cent delta activity. Stage IV contained more than 50 per cent delta activity. Stage REM was characterized by low-voltage, fast-frequency activity, a decrease in the EMG, and large amplitude eye movements. Recording of the EMG and EOG was extremely important in distinguishing epochs of quiet awake, stage I, and stage REM. BM (body movements): When muscle tremor artifact occupied more than two-thirds of an epoch, that epoch was classified as BM. Stage REM: Rapid eye move-

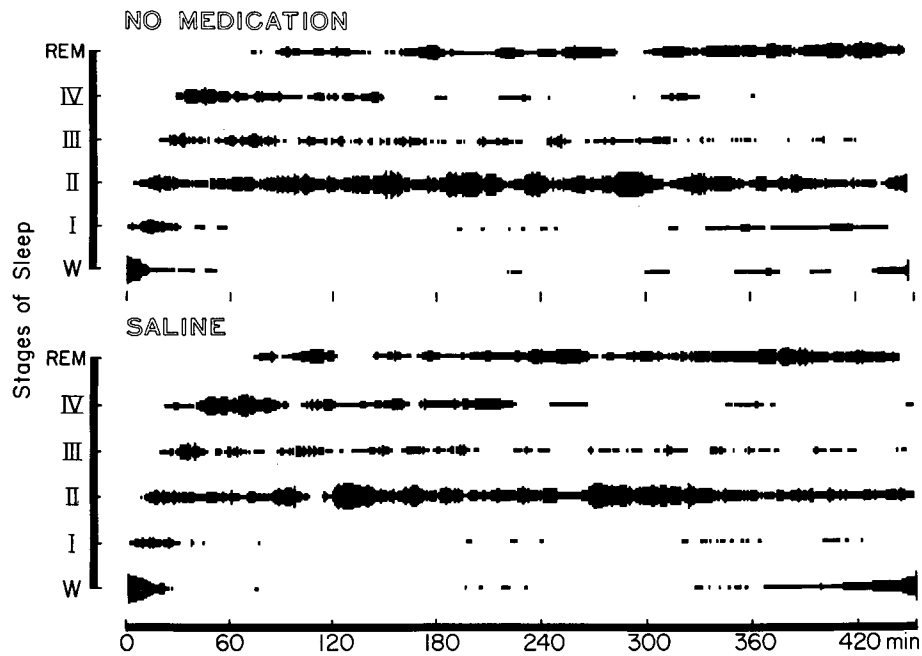

Fig. 1. Effects of saline on the distribution of stages of sleep in man (8 volunteers). On the x axis is plotted the time from onset of recording to awakening 450 minutes later. The y axis shows the number of subjects in a given stage of sleep in which one small square represents one subject. The no medication vs. saline data should be compared visually for each stage. Note that in general there are no marked differences.

Table I. *Effects of single doses of saline, scopolamine, methscopolamine, and chloropromazine on stages of sleep in man*

Drug	N	Stages of sleep (mean per cent ± S.E.)					
		W	*I*	*II*	*III*	*IV*	*REM*
No medication	8	3.9 ± 1.4	5.6 ± 2.3	44.0 ± 2.9	11.5 ± 1.5	8.3 ± 1.3*	20.9 ± 2.0
Saline	8	4.3 ± 1.0	2.8 ± 0.8	39.4 ± 1.3	9.8 ± 1.4	13.7 ± 1.5	22.8 ± 1.6
Scopolamine HBr 0.006 mg. per kilogram	10	3.5 ± 0.8	8.4 ± 1.7*	49.7 ± 1.7‡	9.4 ± 1.2	13.7 ± 1.3	6.9 ± 1.0‡
Methscopolamine Br 0.0055 mg. per kilogram	6	6.0 ± 0.9	3.3 ± 0.8	39.5 ± 2.9	10.5 ± 0.6	11.8 ± 2.3	24.7 ± 1.4
Chlorpromazine HCl 0.4 mg. per kilogram	6	3.6 ± 0.6	2.0 ± 0.2	33.6 ± 1.6*	17.6 ± 2.1†	13.0 ± 1.6	25.7 ± 1.8

*P <0.05.
†P <0.01.
‡P <0.001.

Table II. *Effects of single doses of saline, scopolamine, methscopolamine, and chlorpromazine on mean per cent of NREM and REM sleep, body movements (BM), and number of eye movements in man*

Drug	N	NREM	REM	BM	Total number eye movements
No medication	8	69.6 ± 2.0	20.9 ± 2.0	1.4 ± 0.3	739.2 ± 77.9
Saline	8	66.0 ± 1.9	22.8 ± 1.6	1.1 ± 0.2	635.4 ± 86.9
Scopolamine HBr 0.006 mg. per kilogram	10	81.5 ± 2.3†	6.9 ± 1.0†	4.7 ± 0.9*	114.8 ± 8.0†
Methscopolamine Br 0.0055 mg. per kilogram	6	65.3 ± 0.6	24.7 ± 1.4	1.4 ± 0.4	871.1 ± 95.2
Chlorpromazine HCl 0.4 mg. per kilogram	6	66.5 ± 3.1	25.7 ± 1.8	0.9 ± 0.3	792.1 ± 165.7

*P <0.01.
†P <0.001.

ments during stage REM were defined as such when the deflection in the EOG recording was equal to or greater than 50 μV.

Results

For comparative purposes, the night when saline was injected was used as control in all cases. The duration and distribution of the sleep stages in the saline night were quite similar to the no medication night (Fig. 1). The x axis represents the time from onset of recording time to awakening 450 minutes later. The y axis represents the number of subjects in a given stage of sleep, in which one small square represents one subject. The quantitative data for each stage of sleep are given in Tables I and II. A significant increase in stage IV followed saline as compared to no medication ($p < 0.05$). For this reason, all subsequent data were compared to the saline night.

Effects of scopolamine. The most striking change induced by 0.006 mg. per kilogram of scopolamine HBr was a delay in the onset of REM sleep. As noted in Fig. 2, the onset of stage REM during the

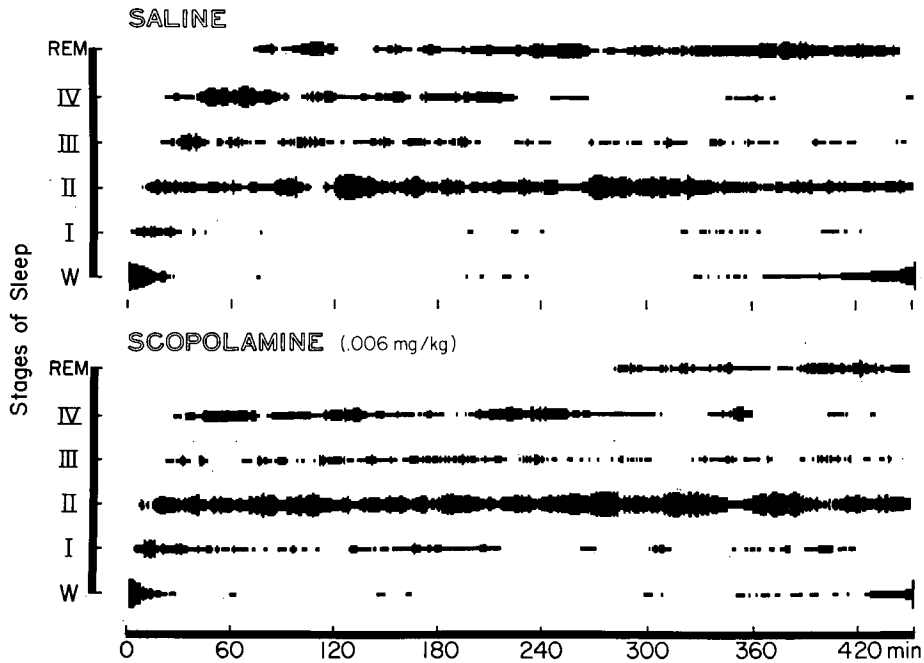

Fig. 2. Effects of scopolamine on the distribution of stages of sleep in man (8 volunteers). The data are plotted as described in Fig. 1. Note that scopolamine (0.006 mg. per kilogram, intramuscularly) caused a dramatic decrease in REM for most of the night. No rebound was observed for the period recorded.

saline night took place during the second hour of sleep (mean time in our series was 105 minutes). In scopolamine-treated individuals there was a complete absence of REM sleep during the first 4½ hours. The mean onset of REM for eight scopolamine-treated subjects occurred 327 minutes after the initiation of recording. Subsequently, bursts of REM were present for the rest of the night (see Fig. 2). As noted in Tables I and II, the total amount of REM stage during the 7½ hours of sleep was significantly reduced in comparison with the saline night ($p < 0.001$). Until the end of the recording period, no rebound in REM was seen. The concomitant increase in non-REM (NREM) sleep appeared mostly as an enhancement of stage II ($p < 0.001$). The duration of stage I was also extended ($p < 0.05$). A marked decrease in the total number of rapid eye movements during the night was observed. Inasmuch as rapid eye movements occur during stage REM, the ratio of eye movements to minutes of stage REM was calculated. This analysis showed that the number of eye movements during stage REM was diminished by the administration of scopolamine (ratio 6.0 for saline and 3.4 for scopolamine; $p < 0.05$). This was also observed when a comparison was made between the total number of rapid eye movements and the epochs of REM in which the rapid eye movements appear (ratio 9.4 for saline and 5.6 for scopolamine; $p < 0.05$). The number of body movements per night was increased by the administration of scopolamine (see Table II).

Effects of methscopolamine. In contrast to the marked changes induced by scopolamine, injection of an equimolar dose of methscopolamine Br did not induce any substantial modification on the pattern of sleep, as noted in Fig. 3 and Tables I and II.

Effects of chlorpromazine. Chlorpromazine in a dose of 0.4 mg. per kilogram did

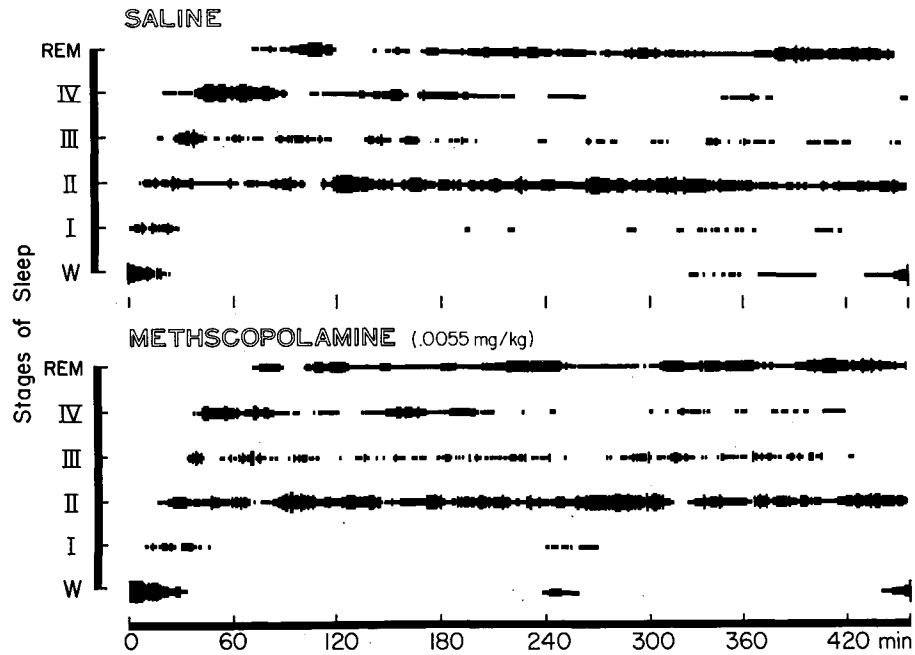

Fig. 3. Lack of effects of methscopolamine on the distribution of stages of sleep in man (6 volunteers). The data are plotted as described in Fig. 1. Note that methscopolamine (0.0055 mg. per kilogram, intramuscularly) did not have any significant effect on REM in contrast to the marked effect of scopolamine (see Fig. 2).

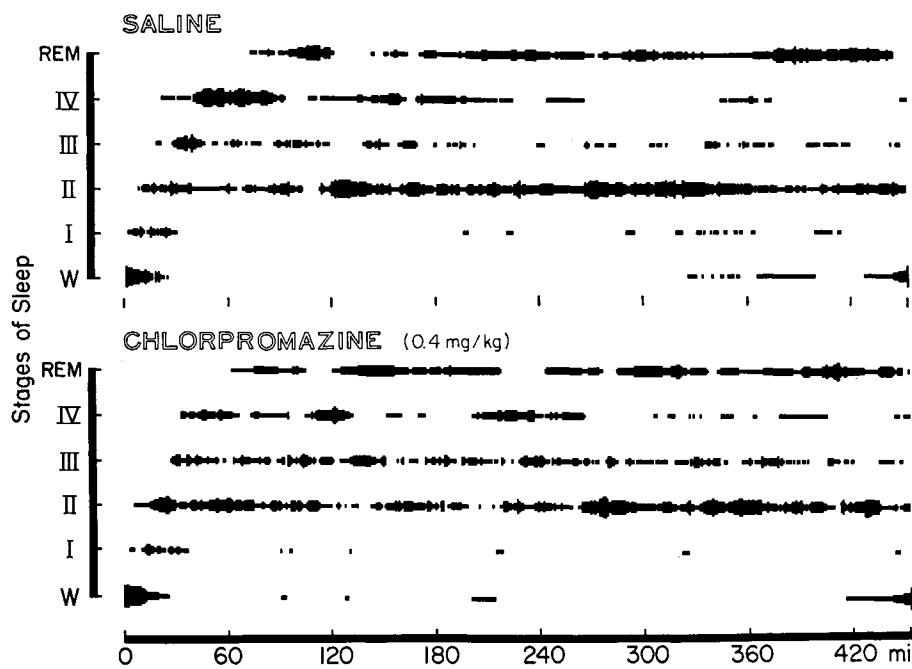

Fig. 4. Effect of chlorpromazine on the distribution of stages of sleep in man (6 volunteers). The data are plotted as described in Fig. 1. Note that chlorpromazine (0.4 mg. per kilogram, intramuscularly) caused an increase in the amount of stage III. In this dose REM was not depressed in contrast to the dramatic effect of scopolamine (see Fig. 2).

not produce any change in the REM/slow wave sleep ratio (see Table II). As noted in Fig. 4, no dramatic changes in the sleep cycle were observed with the possible exception of stage III. Chlorpromazine induced an increase of stage III (from 9.8 per cent in the saline night to 17.6 per cent in the chlorpromazine night; $p < 0.01$). This increase appeared to be at the expense of stage II sleep (which diminished from 39.4 to 33.6 per cent; $p < 0.05$). REM onset was slightly earlier in some subjects than after saline injection, but this was not consistent. The duration of the first period of stage REM was greater after chlorpromazine (mean 30 minutes) than after saline (mean 20.5 minutes). This difference, however, was not statistically significant with the number of subjects studied.

Discussion

The present study clearly shows that a centrally acting muscarinic cholinergic antagonist, scopolamine, induces marked changes in the pattern of sleep in man. REM sleep is clearly reduced, the main effect of scopolamine being to delay its onset. This is counterbalanced by an increase in stages I and II sleep.

In contrast to the dramatic effects of scopolamine, chlorpromazine in similar single doses had negligible effects on REM sleep. An intramuscular dose of 0.4 mg. per kilogram is not large, although it clearly has definite pharmacological effects in man. In this dose it has peripheral α-adrenergic blocking effects and produces sedation. If this dose of chlorpromazine had central adrenergic blocking effects, it would suggest that monoamines are less important to initiating and maintaining REM than is acetylcholine, at least in man. The available evidence in favor of a monoaminergic participation in the mechanisms of REM sleep in the cat is impressive. Destruction of the caudal raphe (whose high concentration in 5HT is well known) is followed by a marked suppression of both NREM and REM sleep.[10] Monoamine oxidase inhibitors markedly suppress REM sleep.[11] This is also the case with inhibitors of norepinephrine and 5HT synthesis.[2, 9]

Evidence in favor of a cholinergic mechanism in REM sleep is growing. Atropine is able to suppress REM sleep in the cat,[8] and pilocarpine enhances stage REM.[18] Furthermore, facilitation of REM sleep is seen in pontine cats after physostigmine,[8] and direct injection of oxotremorine or carbachol into the brainstem reticular formation induces REM sleep in normal cats.[1, 4] Jouvet[9] has interpreted this to mean that acetylcholine may act as a triggering mechanism for adrenergic neurons in REM sleep.

Our results with scopolamine further stress the importance of cholinergic mechanisms in the physiology of sleep in man. The exact nature of this involvement remains to be elucidated. The hypothesis of a triggering mechanism, as suggested by Jouvet, needs direct experimental evidence. Further studies are necessary in order to establish the effects and interactions of drugs with central cholinergic, adrenergic, and serotonergic activity on human sleep.

Our results with chlorpromazine are in accord with those of others[12, 15] using similar doses. There was a lack of correlation between the effects of chlorpromazine and scopolamine upon the sleep patterns, even though their effect on the EEG of awake individuals is relatively similar. Finally, from a clinical point of view, it is interesting to note that scopolamine, often included in sleep-inducing mixtures, does not produce physiological sleep. The fact that most drugs used as soporifics induce a REM-deprived sleep is well known.[14] Since tolerance to REM depression by barbiturates is well known, it would be of interest to determine if similar tolerance to depression of REM occurs with daily doses of scopolamine given at bedtime.

The authors would like to acknowledge the technical assistance of Mr. Gary Gossinger in this study.

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