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Effects of Muscimol or Homotaurine on Sleep–Wake States in Alcohol-Dependent Rats During Withdrawal

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Muscimol Homotaurine $GABA_A$ receptors Sleep–wake states Alcohol-dependence Withdrawal

IMPORTANT alterations of sleep–wake states during the light phase of the nyctohemeral cycle may occur during withdrawal in alcohol-dependent rats (2,25,31). The disorders of sleep–wake states are characterized by an increase of the percentage of both nonactive (NAW) and active wakefulness (AW), and a decrease in slow-wave sleep (SWS) and rapid eye movement sleep (REMS). Among these alterations, the impairment of REMS is the most sensitive (25). Other authors have reported a significant decrease of the activity of $GABA_A$ receptors during alcohol withdrawal (7,10,18,21,22), but the role of $GABA_A$ receptors in sleep mechanisms is also very important: the activation of $GABA_A$ receptors shortens the sleep onset latency, decreases the number of awakenings, and the duration of wakefulness during the night is reduced while total sleep is increased (5). These observations allowed us to study the influence of muscimol and homotaurine on sleep–wake states in alcohol-dependent rats during withdrawal. Muscimol is used in this study, because of its GABAergic agonist pharmacological properties (9,15,20,23,24) and homotaurine, a structural analog of GABA, is considered to be an active GABA mimetic (24,26). Homotaurine acts on the $GABA_A$ receptors, the sites of which are characterized by their affinity for the agonist isoguvacine and their sensitivity to the action of the antagonist bicuculline (6). The aim of this study was to compare the effects of homotaurine and of muscimol on the above-mentioned parameters. To clarify the relationship between alcohol dependence, sleep–wake disturbances and $GABA_A$ receptors, muscimol, and homotaurine were used as pharmacological tools

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to try to normalize these disturbances in sleep–wake states. This last objective was also based on the fact that unlike muscimol, which has toxic properties, homotaurine is well tolerated by the organism. Moreover, a derivative of homotaurine, the calcium acetyl homotaurinate (Ca-AOTA) is used in the treatment of ethanol dependence (4).

METHOD

Animals

Seventy-two male Wistar rats weighing 260–280 g (Iffa Credo Saint Germain sur l'Arbresle, France), were housed in individual cages equipped for automatic and programmed administration of ethanol. Rats were able to move around freely with water and food (standard complete chow, Iffa Credo) available ad lib. The animal room was maintained at a constant temperature (21 ± 1°C) and humidity (60%) with a 12 L:12 D cycle (lights on from 0800–2000 h).

Alcohol Intoxication

All animals were fitted with an indwelling intragastric (IG) catheter under general anesthesia (sodium pentobarbitone, 25 mg/kg, IP) with the distal end cemented to the exterior of the skull (19). Rats were randomly divided into two groups according to type of IG infusion, ethanol rats, and water rats. Ethanol was infused by intragastric administration 1 week after surgery using electronically controlled pumps that were programmed to deliver regular divided doses of 3 ml of solution each administered over 6.5 min. during the 24-h period, at 0430, 0830, 1400, 1900, and 2330 h. Ethanol infusions were 21 ± 2% v/v ethanol–water solutions. The animal was permanently connected to the pump, freedom of movement being assured by a rotating watertight joint inserted between the pump and the infusion catheter. The infused dose represented 10 g absolute ethanol per kg body weight per day (416 mg/kg/h). This amount exceeds the ceiling of metabolic oxidation situated around 300–400 mg/kg/h (27) or 8 mg/kg/ 24 h (12). Moreover, administration in divided doses permits, a relatively constant and sustained blood alcohol level, thus improving the effectiveness of chronic intoxication (8). The duration of intoxication was 13 days, a period sufficient for the development of physical dependence (7,11). Blood alcohol levels were measured on the 2nd and the 12th days of intoxication before the ethanol infusion at 1900 h. Blood was sampled from the tail vein. The ethanol infusions were stopped on the 13th day at 1900 h.

Criteria for classification of intoxication and withdrawal were those determined by Majchrowicz (11): neutrality, sedation, ataxia, and loss of righting reflex for intoxicated animals and general hyperactivity, tremors, induced, or spontaneous convulsions in withdrawn animals.

Normal rats received water by intragastric infusions identical in length of time, volume, and nyctohemeral distribution.

Study of Sleep–Wake States

Electrocortical activity was recorded by electrodes that had been implanted in the same day as the intragastric catheter, on the left frontoparietal surface and electromyographic activity by fitting indwelling electrodes in the nuchal muscle. During the electropolygraphic (EPG) recording of sleep–wake states, the animals could move freely due to rotating commutators attached to the skull microconnector. The reading of EPG data was carried out according to previously published criteria (13,17,28,29). Four types of sleep–wake states were individualized: active wakefulness (AW) corresponding to a state of hypervigilance and active behavior characterized by EEG activity at 7–8 cps with an amplitude greater than 50 μV, and a very high level of EMG activity (coinciding with movement); nonactive wakefulness (NAW) corresponding to calm waking behavior, characterized by EEG activity at 4–7 cps with an amplitude smaller than 50 μV, and a high level of EMG activity, slow–wave sleep (SWS) characterized by EEG activity of 3–5 cps with a large amplitude, and decreased EMG activity, but not having disappeared; and rapid eye movement sleep (REMS), characterized by synchronous cortical activity of 7–8 cps, similar to the AW trace, and no EMG activity. EPG recordings were carried out on withdrawal day between 1100 h (3 h after light) and 1830 (0130 h before darkness), corresponding, respectively, to 1600 and 2330 h after the withdrawal. The recordings time contains the maximal period of AW at the end of the morning (13) and of REMS at the end of the afternoon (17). The recording period is divided into three episodes of short duration: 1) from 1100–1345 h (165 min); 2) from 1400–1645 h (165 min); 3) from 1650–1830 h (100 min), i.e., respectively, from 1600–1845 h, from 1900–2145 h, and from 2150–2330 h after the beginning of the withdrawal. For each episode of short duration, using criteria adapted from Mendelson et al. (16) the following were calculated 1) for each type of sleep–wake state, the percentages, the number, and the mean length of episodes, and 2) the latency of REMS occurrence.

Administration of Substances

On the withdrawal day either physiological saline (10 ml/kg), muscimol (0.25 mg/kg) (Acros, Belgium) or homotaurine (140 mg/kg) (Acros, Belgium) dissolved in physiological saline (0.9%) were administered intraperitoneally (IP). These IP administrations took place at 1030 and 1400 h (i.e., 1530 and 1900 h after withdrawal, respectively). The first administration (i.e., 1030 h) took place half an hour before the EPG recording. The modality of administration and doses of substances were determined based on findings in a previous study (30). Ethanol-treated rats and water-treated rats were divided into six groups of 12 rats receiving ethanol and physiological saline, ethanol and muscimol, ethanol and homotaurine, water and physiological saline, water and muscimol, and water and homotaurine.

Statistical Analysis

The results of sleep–wake states were evaluated using the analysis of variance (ANOVA), including factors "substance," "alcohol," and "repetitive measures" over time (three episodes) to study changes occurring during EPG recordings period. ANOVA, when significant, was followed by Scheffé test to compare effects of substances in each recording episode. Student's *t*-test was used to compare intoxicated and nonintoxicated rats in each group receiving a substance (i.e., saline, muscimol, or homotaurine). Values of body weight have been compared using the ANOVA test. All the results are expressed as the mean ± SEM. Differences were considered significant at $p < 0.05$.

RESULTS

Evaluation of Alcohol Intoxication

Measurement of blood alcohol and weight. The mean blood alcohol level in the second day of the intoxication period was 1.47 ± 0.08 mg per 100 ml (mean ± SEM) and in the 12th day

1.51 ± 0.09 mg per 100 ml. Body weight measured on the 1st, 3rd, 10th, and last day of intoxication showed a regular increase that was similar in both groups, 242.92 ± 3.12g to 277.78 ± 5.89g in ethanol rats, and 239.58 ± 3g to 272.78 ± 3.68g in water rats. Thus, demonstrating that nutrition was satisfactory in the rats intoxicated with alcohol [ANOVA $F(1, 283) = 2.33$, $p = 0.128$].

Observation of general behavior. Observation was carried out: 1) during the period of intoxication under the direct effect of ethanol; 2) during withdrawal at 0830, 1030, 1400, and 1830 h, i.e., 1330, 1530, 1900, and 2330 h after termination of ethanol treatment. This demonstrated that under the direct effect of ethanol intoxication, the signs of acute intoxication were present in all animals (loss of the righting reflex, ataxia, etc.) that are normally observed after ethanol infusions. During withdrawal, the presence of intense signs of withdrawal were noted in all animals (general hyperactivity, piloerection, tail spasticity, tail tremor, or generalized) 1330 and 1530 h after the last alcohol infusion.

Study of sleep–wake states (Tables 1, 2, 3, and 4)

In the alcohol-dependent rat during withdrawal vs. water rats receiving physiological saline, there was an increase in the percentages of active wakefulness and nonactive wakefulness, and a decrease in slow-wave sleep and rapid eyes movement sleep. However, the most significant time in our study is situated between 1400 and 1645 h, the second episode of recording, i.e., 1900 to 2145 h after the beginning of withdrawal, in which all these modifications were observed (Table 2). From 1100–1345 h (Table 1), the first episode of recording, there is an increase in the active and nonactive wakefulness and a decrease in the slow-wave sleep; from 1650–1830, the third episode of recording (Table 3), the main perturbations concerned rapid eye movement sleep, with the decrease in the percentage, and the shortening of its latency of occurrence.

For muscimol. During the first 165 min (from 1100–1345 h; Table 1), we observed a decrease in the percentage of active wakefulness to a value close to that seen in the nonalcohol-dependent rat that received saline solution [ANOVA, $F(2, 139) = 3.014$, $p = 0.05$, and Scheffé test, $p < 0.05$]; also, there was a tendency to increase the percentages of slow-wave sleep and rapid eyes movement sleep, which was not significant. During the following 165 min (from 1400–1645 h; Table 2) we observed a decrease in percentage of nonactive wakefulness [ANOVA, $F(2, 210) = 7.707$, $p = 0.001$, and Scheffé test, $p < 0.05$]; an increase in the percentages of slow-wave sleep [ANOVA, $F(2, 210) = 4.396$, $p = 0.013$, and Scheffé test, $p < 0.05$]; and rapid eyes movement sleep [ANOVA, $F(2, 210) = 4.355$, $p = 0.014$, and Scheffé test: $p < 0.05$]. During the last 100 min of the recordings (from 1650–1830 h; Table 3) there was no significant difference in sleep–wake states. For the whole light phase recording (from 1100–1830 h; Table 4) there was a decrease in percentages of active wakefulness [ANOVA, $F(2, 33) = 4.33$, $p = 0.021$, and Scheffé test, $p < 0.05$] and nonactive wakefulness [ANOVA, $F(2, 68) = 10.488$, $p = 0.0001$, and Scheffé test, $p < 0.05$]; an increase in the percentages of slow-wave sleep [ANOVA, $F(2, 68) = 7.677$, $p = 0.001$, and Scheffé test, $p < 0.05$] and rapid eye movement sleep [ANOVA, $F(2, 68) = 8.543$, $p = 0.0001$, and Scheffé test, $p < 0.05$]; and a decrease in the mean length of episodes of nonactive wakefulness [ANOVA, $F(2, 68) = 6.962$, $p = 0.002$, and Scheffé test, $p < 0.05$].

For homotaurine. During the first 165 min (from 1100–1345 h; Table 1), there was a decrease in the percentage of nonactive wakefulness [ANOVA, $F(2, 210) = 7.707$, $p = 0.001$, and Scheffé test, $p < 0.05$] and an increase in the percentage of slow-wave sleep and rapid eye movement sleep, which were not significant. During the following 165 min (from 1400–1645 h; Table 2) a decrease in the percentage of active wakefulness [ANOVA, $F(2, 139) = 3.014$, $p = 0.05$, and Scheffé test, $p < 0.05$], and an increase in the percentage of

TABLE 1

ELECTROPOLYGRAFIC DATA (MEAN ± SEM) FROM 1100 h TO 1345 h IN THE ETHANOL AND WATER RATS RECEIVING DURING WITHDRAWAL 0.9% SALINE SOLUTION, MUSCIMOL, OR HOMOTAURINE

	Saline		Muscimol		Homotaurine	
	Ethanol	Water	Ethanol	Water	Ethanol	Water
Active wakefulness (AW)						
Percentage	20.87 ± 2.16	15.40 ± 1.41*	13.50 ± 2.16	15.07 ± 2.38	20.08 ± 2.54	13.07 ± 1.58*
Number of episodes	17.83 ± 2.20	13.75 ± 1.74	13.67 ± 0.13	14.67 ± 3.10	13.08 ± 2.04	11.42 ± 1.71
Mean duration of episodes (min)	2.05 ± 0.13	1.98 ± 0.20	1.77 ± 2.20	2.02 ± 0.22	3.12 ± 0.42	2.58 ± 0.72
Nonactive wakefulness (NAW)						
Percentage	41.39 ± 2.11	30.07 ± 2.47†	38.37 ± 2.82	26.36 ± 2.00‡	33.17 ± 2.10	28.10 ± 2.48
Number of episodes	45.92 ± 3.29	42.83 ± 2.89	52.50 ± 3.14	47.75 ± 3.40	43.67 ± 2.09	40.58 ± 2.97
Mean duration of episodes (min)	1.59 ± 0.15	1.24 ± 0.16	1.22 ± 0.13	0.94 ± 0.07*	1.28 ± 0.09	1.19 ± 0.11
Slow wave sleep (SWS)						
Percentage	34.85 ± 3.32	50.78 ± 3.31‡	44.03 ± 3.44	53.47 ± 3.23*	43.03 ± 3.36	54.10 ± 2.42‡
Number of episodes	34.25 ± 3.88	33.75 ± 2.77	43.50 ± 3.17	39.00 ± 2.70	34.42 ± 2.27	36.67 ± 1.84
Mean duration of episodes (min)	1.80 ± 0.25	2.70 ± 0.27‡	1.64 ± 0.10	2.42 ± 0.23‡	2.09 ± 0.11	2.53 ± 0.17*
Rapid eyes movements sleep (REMS)						
Percentage	2.95 ± 0.73	3.72 ± 0.55	4.10 ± 0.88	5.08 ± 0.66	4.55 ± 0.86	4.72 ± 0.45
Number of episodes	6.25 ± 1.81	6.42 ± 1.10	7.17 ± 1.64	7.33 ± 1.14	7.17 ± 1.13	8.83 ± 1.45
Mean duration of episodes (min)	0.90 ± 0.21	1.20 ± 0.16	0.86 ± 0.11	1.25 ± 0.19	1.09 ± 0.09	1.10 ± 0.15
Latency of occurence	78.73 ± 8.90	78.72 ± 8.87	78.30 ± 12.42	85.33 ± 10.97	85.64 ± 9.93	74.88 ± 6.64
Number of stage changes	104.25 ± 7.49	97.25 ± 7.00	116.83 ± 7.14	108.75 ± 5.95	98.25 ± 4.74	97.50 ± 4.59

*$p \leq 0.05$; †$p \leq 0.001$; ‡$p \leq 0.01$; Student's *t*-test, water vs. ethanol.

TABLE 2

ELECTROPOLYGRAFIC DATA (MEAN ± SEM) FROM 1400 TO 1645 h, IN THE ETHANOL AND WATER RATS, RECEIVING DURING WITHDRAWAL 0.9% SALINE SOLUTION, MUSCIMOL, OR HOMOTAURINE

	Saline		Muscimol		Homotaurine	
	Ethanol	Water	Ethanol	Water	Ethanol	Water
Active wakefulness (AW)						
Percentage	16.45 ± 1.35	11.08 ± 1.60‡	13.47 ± 2.00	9.10 ± 1.81	11.10 ± 1.62	11.12 ± 0.90
Number of episodes	9.75 ± 0.84	9.25 ± 1.18	8.67 ± 1.14	7.83 ± 1.42	7.08 ± 1.07	6.75 ± 0.78
Mean duration of episodes (min)	2.92 ± 0.22	2.05 ± 0.20‡	3.15 ± 0.74	1.84 ± 0.25	2.75 ± 0.25	3.15 ± 0.55
Nonactive wakefulness (NAW)						
Percentage	36.17 ± 2.03	24.20 ± 2.64†	24.83 ± 3.24	19.68 ± 1.83	30.53 ± 2.84	21.82 ± 1.80‡
Number of episodes	45.25 ± 2.89	38.58 ± 4.04	44.92 ± 2.33	35.08 ± 2.57‡	41.83 ± 1.87	38.00 ± 3.40
Mean duration of episodes (min)	1.39 ± 0.11	1.17 ± 0.21	1.02 ± 0.15	0.95 ± 0.09	1.23 ± 0.13	0.98 ± 0.05
Slow wave sleep (SWS)						
Percentage	43.02 ± 2.91	55.37 ± 2.96‡	53.72 ± 3.59	62.33 ± 2.85	49.38 ± 3.12	58.59 ± 1.37‡
Number of episodes	40.25 ± 2.77	39.00 ± 2.79	47.08 ± 3.16	38.83 ± 1.66*	44.58 ± 2.93	39.67 ± 2.83
Mean duration of episodes (min)	1.97 ± 0.32	2.68 ± 0.36	1.91 ± 0.17	2.71 ± 0.16*	1.86 ± 0.10	2.69 ± 0.31*
Rapid eyes movements sleep (REMS)						
Percentage	5.20 ± 0.59	9.35 ± 0.89†	7.98 ± 0.87	8.97 ± 0.85	9.38 ± 1.19	8.46 ± 0.75
Number of episodes	9.08 ± 1.44	13.75 ± 1.70*	15.42 ± 1.61	14.75 ± 1.81	14.50 ± 2.39	13.33 ± 1.98
Mean duration of episodes (min)	1.16 ± 0.13	1.20 ± 0.11	0.86 ± 0.07	1.05 ± 0.07*	1.27 ± 0.13	1.17 ± 0.10
Latency of occurence	58.31 ± 9.23	47.53 ± 8.69	32.55 ± 3.40	40.25 ± 7.18	35.97 ± 4.82	51.36 ± 21.80*
Number of stage changes	104.33 ± 6.89	100.58 ± 7.40	116.08 ± 6.34	96.50 ± 3.97*	108.00 ± 4.93	97.75 ± 5.62

*$p \leq 0.05$; †$p \leq 0.001$; ‡$p \leq 0.01$; Student's *t*-test, water vs. ethanol.

rapid eye movement sleep [ANOVA, $F(2, 210) = 4.355$, $p = 0.014$, and Scheffé test, $p < 0.05$] was seen. During the last 100 min of the recordings (from 1650–1830 h; Table 3) there was a tendency to increase the percentage of rapid eye movement sleep, which was not significant. For the whole light phase recording (from 1100–1830 h.; Table 4) a decrease in the percentage of nonactive wakefulness [ANOVA, $F(2, 68) = 10.488$, $p = 0.0001$, and Scheffé test, $p < 0.05$]; an increase in the percentages of slow-wave sleep [ANOVA, $F(2, 68) = 7.677$, $p = 0.001$, and Scheffé test, $p < 0.05$] and rapid eye movement sleep [ANOVA, $F(2, 68) = 8.543$, $p = 0.0001$, and Scheffé test, $p < 0.05$] was observed.

TABLE 3

ELECTROPOLYGRAFIC DATA (MEAN ± SEM) FROM 1650 TO 1830 h, IN THE ETHANOL AND WATER RATS RECEIVING DURING WITHDRAWAL 0.9% SALINE SOLUTION, MUSCIMOL, OR HOMOTAURINE

	Saline		Muscimol		Homotaurine	
	Ethanol	Water	Ethanol	Water	Ethanol Rats	Water Rats
Active wakefulness (AW)						
Percentage	7.75 ± 1.77	10.72 ± 6.57	9.36 ± 3.22	14.22 ± 2.76	11.67 ± 8.56	9.35 ± 1.75
Number of episodes	3.67 ± 0.50	3.42 ± 0.74	3.08 ± 0.70	6.17 ± 1.19*	4.67 ± 0.75	3.58 ± 0.58
Mean duration of episodes (min)	2.20 ± 0.41	3.47 ± 0.71	2.35 ± 0.68	3.21 ± 1.08	2.55 ± 0.50	2.96 ± 0.55
Nonactive wakefulness (NAW)						
Percentage	24.42 ± 1.94	20.36 ± 2.76	21.18 ± 2.27	18.03 ± 2.39	18.29 ± 2.06	17.02 ± 1.16
Number of episodes	23.75 ± 1.95	17.08 ± 2.01*	27.25 ± 2.11	21.50 ± 2.26	20.58 ± 1.40	20.08 ± 1.54
Mean duration of episodes (min)	1.16 ± 0.19	1.31 ± 0.20	0.84 ± 0.10	0.93 ± 0.08	0.96 ± 0.13	0.98 ± 0.11
Slow wave sleep (SWS)						
Percentage	57.11 ± 2.85	54.25 ± 3.11	57.24 ± 4.58	55.66 ± 2.95	55.92 ± 2.47	58.06 ± 2.07
Number of episodes	29.08 ± 2.52	24.50 ± 1.74	31.17 ± 2.07	23.58 ± 1.20†	24.25 ± 1.67	25.67 ± 1.26
Mean duration of episodes (min)	2.20 ± 0.29	2.38 ± 0.26	1.98 ± 0.20	2.55 ± 0.18*	2.55 ± 0.20	2.65 ± 0.20
Rapid eyes movements sleep (REMS)						
Percentage	10.72 ± 1.05	15.14 ± 1.45†	12.22 ± 1.01	11.47 ± 1.42	14.12 ± 1.38	15.56 ± 1.18
Number of episodes	12.00 ± 1.78	12.50 ± 1.21	11.83 ± 0.91	10.58 ± 1.41	11.67 ± 1.13	13.92 ± 1.26
Mean duration of episodes (min)	1.19 ± 0.15	1.20 ± 0.09	1.10 ± 0.08	1.14 ± 0.08	1.31 ± 0.11	1.36 ± 0.16
Latency of occurence	9.86 ± 2.21	19.28 ± 4.75*	7.17 ± 1.15	13.28 ± 3.81	7.52 ± 2.06	10.36 ± 1.86
Number of stage changes	68.50 ± 5.84	57.58 ± 3.80	73.33 ± 4.01	61.83 ± 2.58*	61.17 ± 3.46	63.25 ± 2.83

*$p \leq 0.05$; †$p \leq 0.01$; Student's *t*-test, water vs. ethanol.

TABLE 4

WHOLE ELECTROPOLYGRAFIC DATA (MEAN ± SEM) FROM 1100 TO 1830 h, IN THE ETHANOL AND WATER RATS, RECEIVING DURING WITHDRAWAL 0.9% SALINE SOLUTION, MUSCIMOL, OR HOMOTAURINE

	Saline		Muscimol		Homotaurine	
	Ethanol	Water	Ethanol	Water	Ethanol	Water
Active wakefulness (AW)						
Percentage	16.76 ± 0.93	12.50 ± 0.68‡	12.11 ± 0.98	12.80 ± 1.46	15.39 ± 1.54	11.18 ± 0.77*
Number of episodes	31.25 ± 2.32	26.42 ± 2.29	25.42 ± 3.34	28.83 ± 3.38	24.83 ± 2.76	22.17 ± 2.60
Mean duration of episodes (min)	2.29 ± 0.11	2.17 ± 0.18	2.29 ± 0.31	2.02 ± 0.18	2.88 ± 0.31	2.53 ± 0.30
Nonactive wakefulness (NAW)						
Percentage	34.76 ± 0.97	25.53 ± 1.65§	28.13 ± 1.19	21.37 ± 1.19†	27.33 ± 1.59	22.32 ± 1.32*
Number of episodes	114.08 ± 7.09	93.83 ± 3.73	127.67 ± 3.73	104.33 ± 6.12‡	106.08 ± 4.24	98.58 ± 6.71
Mean duration of episodes (min)	1.38 ± 0.10	1.31 ± 0.16	1.00 ± 0.06	0.91 ± 0.06	1.15 ± 0.09	1.03 ± 0.07
Slow wave sleep (SWS)						
Percentage	43.42 ± 1.80	53.45 ± 1.85†	51.67 ± 1.64	57.11 ± 1.96*	48.47 ± 2.01	56.91 ± 1.27‡
Number of episodes	104.42 ± 7.32	101.42 ± 4.47	121.75 ± 5.08	101.42 ± 4.31‡	103.25 ± 4.93	101.17 ± 4.75
Mean duration of episodes (min)	1.97 ± 0.26	2.34 ± 0.15	1.89 ± 0.09	2.52 ± 0.14†	1.92 ± 0.16	2.55 ± 0.16‡
Rapid eyes movements sleep (REMS)						
Percentage	5.68 ± 0.56	8.51 ± 0.37‡	8.10 ± 0.49	8.51 ± 0.62	9.35 ± 0.75	9.58 ± 0.37
Number of episodes	27.33 ± 4.34	32.67 ± 2.15	34.42 ± 2.49	32.67 ± 3.39	33.33 ± 3.58	36.17 ± 3.58
Mean duration of episodes (min)	1.07 ± 0.11	1.18 ± 0.08	1.06 ± 0.06	1.20 ± 0.08	1.31 ± 0.09	1.26 ± 0.10
Number of stage changes	277.08 ± 17.68	254.33 ± 13.19	306.25 ± 11.3	267.25 ± 8.71*	269.58 ± 10.78	258.50 ± 14.56

*$p \leq 0.05$; †$p \leq 0.001$; ‡$p \leq 0.01$; §$p < 0.0001$; Student's t-test, water vs. ethanol.

In nonalcohol-dependent rats, muscimol, and homotaurine had no significant effect on sleep–wake states.

DISCUSSION

Our results show that muscimol induces significant modifications in the sleep–wake states in the alcohol-dependent rats during withdrawal, in the light phase of the nyctohemeral cycle. The relationship between these disturbances and alcohol-dependent rats during withdrawal have been discussed in another study (25). These changes are most remarkable during the first three quarters of the duration of the light phase. Muscimol influences all four sleep–wake states, which have a tendency to normalize their percentages close to those encountered in the nonalcohol-dependent rats who received 0.9% saline solution. This evolution of the sleep–wake states mainly affects the active wakefulness, which decreases, and especially for REMS, which constantly increases, but its latency remained shortened. It is important to stress that the temporal relationship observed following the administration of muscimol and homotaurine and the effects of these drugs on sleep–wake stages could be determined by the ability of each drug to cross the blood–brain barrier, and also by their plasma half-life (3). Taking into account this last parameter, we established the intervals between the drug administrations, to have a plasma level that remained as constant as possible throughout the experiment.

Homotaurine does not significantly alter active wakefulness during the whole of the light phase of the nyctohemeral cycle. However, during the middle part of this period (between 1400–1645 h) we noticed a trend towards a decrease in active wakefulness. It also caused a slight decrease in nonactive wakefulness during the whole of the light phase, this tendency being strongest during the first 165 min of the recordings. As muscimol, homotaurine also increased the percentages of SWS and REMS, without affecting its latency, during the whole duration of the light phase of the nyctohemeral cycle of the alcohol-dependent rats.

It is important to note neither muscimol or homotaurine significantly influenced the sleep–wake states in the nonalcohol-dependent rats. This is consistent with other author's observations on the role of $GABA_A$ receptors in the mechanisms of sleep in the normal rat (14).

Our data show that the administration of $GABA_A$ agonists in alcohol-dependent rats significantly affected the sleep–wake states during withdrawal, probably by increasing the activity of these receptors, which is decreased during ethanol withdrawal (7,10,18,21), and which, in turn, could modulate the activity of other neurotransmitter systems involved in sleep–wake states, such as brain biogenic monoamines and acetylcholine (1,9). This hypothesis is also supported by the fact that, except for REMS, the influence of these drugs on the sleep–wake states is different during different sequences of the light phase of nyctohemeral cycle.

Another important observation is that the influence of muscimol and homotaurine on sleep–wake states, though quite similar, is not identical, being more important for muscimol, which is a direct agonist of $GABA_A$ receptors. This difference could be due to the different mechanisms of interaction with these receptors, for the two molecules.

CONCLUSIONS

The agonists of $GABA_A$ receptors, muscimol, and homotaurine, significantly influenced sleep–wake states in alcohol-dependent rats during withdrawal, but not in the nonalcohol-dependent rats, during the light phase of the nyctohemeral cycle. These influences are probably due to the stimulation of the $GABA_A$ receptors, which have a decreased activity during the withdrawal in alcohol-dependent rats. The stimulation of the depressed $GABA_A$ receptors may possibly modulate neurotransmitter systems involved in the generation of sleep–wake states, which are impaired during withdrawal in alcohol-dependents rats.

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