EFFECT OF DELTA SLEEP-INDUCING PEPTIDE ON ELECTROPHYSIOLOGICAL PARAMETERS OF SLEEP IN RATS DURING ABSTINENCE FROM ALCOHOL

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There is experimental and clinical evidence of the essential role of disturbances of the sleep – waking process in the structure of the alcohol abstinence syndrome [6, 7, 9]. Hence the urgent importance of a preclinical search for pharmacological correctors of this process in the period of withholding alcohol in animals with a developed pathological craving for ethanol.

The aim of this investigation was to study the action of delta sleep-inducing peptide (DSIP) on several parameters of the electrophysiological structure of sleep during the period of abstinence from alcohol in rats.

EXPERIMENTAL METHOD

Noninbred male albino rats with a stable and high mean daily level of consumption of 15% ethanol solution (body weight at the beginning of the investigation 180-200 g) were chosen for the experiments. Initially the animals were given alcohol in communal cages in the form of 0.1% saccharine solution in 15% ethanol daily for 3 months with the aim of developing a stable alcohol motivation in them. Saccharine was added to improve the taste of the alcohol solution. Next the rats were placed in individual cages and for 10 days the level of their mean daily consumption of sweet ethanol was recorded while free access was allowed to the alcohol solution and water. To avoid the development of an increased addiction to alcohol, because of being kept in isolation (isolation stress) [1] the animals were deprived of access to alcohol for 3 days and kept in groups. After deprivation of alcohol for 3 days the rats were returned to the individual cages with two measuring bowls containing water and sweet ethanol solution, and the level of consumption of single doses of alcohol solution was recorded for 1.5 h under conditions of "alcohol thirst." For the next experiments animals with a high stable alcohol consumption, both mean daily and sessional, were selected. The animals were then retested in individual cages, the sweet solution of 15% ethanol being replaced by unsweetened, so that rats consuming sweet ethanol only because of its improved taste could be excluded from the future experiments. The procedures of selection of the animals described above made it possible to discover individuals with a developed and stable pathological addiction, purely for ethanol. These rats continued to receive a 15% solution of ethanol for 10 months while being kept under ordinary conditions and provided with the necessary amounts of dry food and water. The animals then underwent an operation to permit further electrophysiological recording of their sleep structure [3]. Between 7 and 10 days after the operation on the animals, they were placed for 3-4 days in transparent plastic chambers at the same time of day (from 12 noon to 4 p.m.) in order to adapt them to the subsequent experiments, after which their electrophysiological sleep parameters were recorded continuously for 4 h against the background of voluntary ethanol consumption, on a 17-channel polygraph (Nihon Kohden, Japan). The animals were then deprived of alcohol and their sleep was recorded 24 and 48 h after withdrawal of the ethanol, i.e., during alcohol abstinence. Immediately thereafter the animals again received alcohol for 10-15 days and, after withdrawal of alcohol from them once again, the effect of DSIP on the sleep – waking process was studied at the same times of the alcohol abstinence

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TABLE 1. Changes in Sleep Structure of Rats (in min) during Alcohol Withdrawal (effect of DSIP, 0.1 mg/kg, on sleep of animals during alcohol abstinence

Animal	Exptl. conditions	TDS	SSP	REM sleep	Number of episodes of REM sleep	Mean duration of episodes of REM sleep	Latent period of REM sleep	Onset of complete SSP-REM sleep cycle	Mean sleep duration w/o awakening during TDS	Mean sleep duration w/o awakening after latent REM period	Duration of wakening
With relative deficit of REM sleep against a background of alcoholization (n = 5)	I	146	143	3	4	0,8	57	103	1,6	3,2	100
	II	88*	87*	1,3*	1*	1,3*	80	216*	1,3	1,4	152*
	III	101	96*	4,6	4	1,2	54	147	1,9	1,4	136
	IV	107	104	3,2	3	1,1	82	200*	2,5	2,6	134
	V	190*	164	26*	14*	1,9*	42	57*	4,6*	2,6	51*
With relative excess of REM sleep against a background of alcoholization (n = 8)	I	158	143	15	12	1,2	10	15	2,3	2,7	83
	II	151	134	17	10	1,8	22	49	2,7	2,4	89
	III	135	118	16	9	1,8	9	19	2,8	1,8	105
	IV	167	161	6*	5*	1,1	80*	133*	3,2*	2,6	73
	V	173*	160*	13	9	1,4	38*	54	3,5*	2,9	67

Legend. I) Sleep structure of rats against a background of alcoholization; II) animals; sleep 24 h after withdrawal of alcohol; III) animals' sleep 48 h after withdrawal of alcohol; IV) DSIP 1 h before recording of sleep, 24 h after withdrawal of alcohol; V) DSIP 1 h before recording of sleep 48 h after withdrawal of alcohol. Asterisk indicates results statistically significant at the $p < 0.05$ level; n) number of animals.

period. The peptide was injected intraperitoneally in a dose of 0.1 mg/kg 1 h before electrophysiological recording of sleep. The results were subjected to statistical analysis by the nonparametric differences test [8] and also by comparison of means by the T test [2].

EXPERIMENTAL RESULTS

Selection of the animals in accordance with the scheme described above enabled individuals with a combination of high and stable consumption of 15% ethanol solution, both expressed as the mean daily consumption and as the single sessional consumption (after its deprivation), as a result of a period of alcoholization for 13 months, to be distinguished. The values of these parameters were 32.9 ± 4.3 and 3.6 ± 0.8 ml/kg respectively.

Our previous investigations showed that the procedure of administration of sweet ethanol for 2-3 months is sufficient to distinguish individuals with a developed pathological craving for ethanol for experiments to identify and conduct a preclinical study of substances for the pharmacotherapy of alcoholism [5]. The switching of the animals after this procedure to consumption of unsweetened alcohol solution and additional subsequent alcoholization for 10 months suggests that these individual rats are dependent on alcohol, and in turn this enables the mechanisms of this dependence and the spectrum of pharmacological activity of potential antialcohol remedies to be studied.

In rats with chronically implanted electrodes for electrophysiological recording of the sleep structure differences were found of a quantitative nature between the proportions of its individual phases (Table 1). For instance, against the background of voluntary alcohol consumption individuals were discovered with relative insufficiency of the REM phase of sleep, when REM sleep accounted on average for 2% of the total duration of sleep (TDS). There were also some rats in which REM sleep accounted for 12% or more of TDS. It is interesting to note that withdrawal of alcohol caused opposite changes in the quantitative representation of these phases: in animals with an initial deficit it was curtailed even more, whereas in rats with an increased contribution, the proportion of this phase increased, i.e., a "rebound phenomenon" of REM sleep probably took place, which is often a reflection of the alcohol abstinence syndrome [6, 9]. The slow sleep phase (SSP) in these animals was depressed both 24 and 48 h after alcohol deprivation, and at the same time the duration of waking rose sharply on account of the number of awakenings and, especially, an increase in their mean duration. Reduction of the depth of sleep also was characterized by shortening of the average sleep time without awakenings during the whole period of recording. Worsening of the process of falling asleep was revealed by an increase in the time of onset of the first complete SSP – REM sleep cycle.

Thus the period of alcohol withdrawal was accompanied by profound disturbances in the electrophysiological structure of sleep and by abrupt changes in the quantitative relations between individual stages of this process; this may probably be further evidence that the individuals studied were physically dependent on ethanol.

The next step of the investigation was to study the effect of DSIP (0.1 mg/kg, intraperitoneally, 1 h before recording) on the electrophysiological parameters of the rats' sleep during the periods of alcohol deprivation studied previously (after 24 and 48 h). A considerable increase in TDS was found as early as after the first injection of the neuropeptide, and mainly due to an increase in SSP, curtailment of the waking time, duration of individual awakenings, and an increase in the depth of sleep. REM sleep which, as is generally considered, compensates for emotional-psychological losses during waking [4], underwent various changes under the influence of DSIP. In rats with inhibited REM sleep in the period of alcohol abstinence, the neuropeptide facilitated an increase in its duration on account of the number of separate episodes and their mean duration. This was observed to the greatest degree after a second injection, i.e., 48 h after withdrawal of alcohol. In the animals characterized by a so-called "REM sleep rebound phenomenon," at this period, DSIP, on the contrary, reduced somewhat the quantitative representation of this phase, bringing it back toward the physiological normal. This effect was manifested to the greatest degree 48 h after withdrawal of alcohol also.

Thus DSIP regulates the sleep — waking process and disturbances during the period of alcohol abstinence. On the basis of the results of this investigation the peptide can be recommended for correcting manifestations of the alcohol abstinence syndrome and alcohol-induced delirium in the electrophysiological sleep structure of patients with alcoholism.

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