5. Yu. D. Ignatov, A. T. Kachan, and Yu. N. Vasil'ev, Acupuncture Analgesia [in Russian], Leningrad (1990).
6. L. V. Kalyuzhnyi, Physiological Mechanisms of Regulation of Pain Sensitivity [in Russian], Moscow (1984).
7. Yu. N. Limanskii, Physiology of Pain [in Russian], Kiev (1986).
8. V. A. Mikhailovich and Yu. D. Ignatov, The Pain Syndrome [in Russian], Leningrad (1990).
9. A. P. Myasnikov, Medical Service for Divers, Aquaplane Enthusiasts, and Caisson Workers [in Russian], Leningrad (1977).
10. I. A. Sapov, A. S. Solodkov, V. Ya. Nazarkin, et al., Physiology and Pathology of Diving and Safety Measures on Water [in Russian], Moscow (1986).
11. A. I. Basbaum and H. L. Fields, Ann. Rev. Neurosci., 7, 309 (1984).
12. J. A. Best, D. C. Chen, L. K. Vaugh, et al., FASEB J., 3, No. 3, 421 (1989).
13. A. W. Duggan, Phil. Trans. R. Soc. London, B-308, 375 (1985).
14. M. J. Millan, Pain, 27, 303 (1986).

EFFECT OF PROPRANOLOL ON CIRCADIAN RHYTHM OF DURATION OF THE HYPNOGENIC ACTION OF DIAZEPAM AND HEXOBARBITAL

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The hypnogenic effect of general anesthetics and sedatives varies in its intensity throughout the 24-h period [8]. The time course of pharmacogenic sleep is significantly affected by administration of melatonin [1], the principal hormone of the pineal gland, which is involved in the organization of circadian rhythms [9]. Nervous control of the activity of this cerebral gland depends entirely on a sympathetic nerve, which has beta-adrenoreceptors distributed on its endings. The search for ways of controlling deep pharmacogenic sleep through modulation of pineal activity is an interesting problem.

In the investigation described below the character of the action of the beta-adrenoblocker propranolol on the circadian rhythm of the effect of diazepam and hexobarbital was studied.

EXPERIMENTAL METHOD

Altogether 48 groups of experiments were carried out on 288 male albino mice weighing 20-30 g, during May. The animals were kept under standard conditions (as regards number to a cage, diet, ambient temperature), with a fixed ratio (1:1) of light and darkness, the period of light being from 8 a.m. to 8 p.m. The latent period of assumption of the side position by the animals, and its duration, after injection of a standard dose of diazepam (50 mg/kg, intraperitoneally – the other drugs in the same way) and of hexobarbital (75 mg/kg) were estimated. In the experimental series, administration of these substances was preceded (30 min previously) by injection of propranolol (5 mg/kg), but in the control series by injection of the same volume of physiological saline. The results were subjected to statistical analysis by Student's test and the Wilcoxon–Mann–Whitney test.

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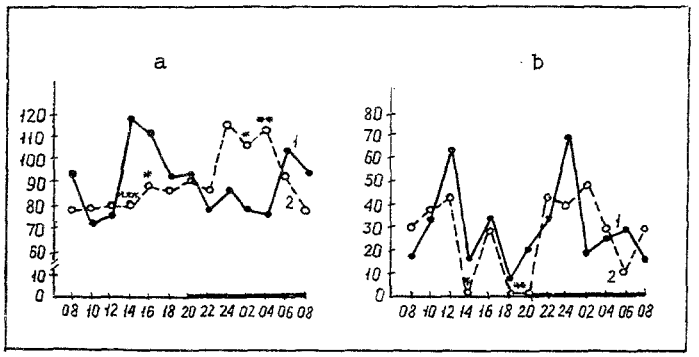

Fig. 1. Changes in circadian rhythm of duration of hypnogenic effect of diazepam (a) and hexobarbital (b) under the influence of propranolol. 1) Results of control determinations (combination of substances with injection of physiological saline); 2) injection of drug preceded by propranolol. Each point on curve indicates results of a separate group of experiments (on six mice). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ differences statistically significant. Abscissa, clock time; ordinate, duration of pharmacogenic sleep at different times of the 24-h period (in min).

EXPERIMENTAL RESULTS

In the two control series the circadian fluctuations of the duration of the hypnogenic effect of diazepam and hexobarbital alone were first determined. For this purpose the action of the drug was monitored every 2 h throughout the complete 24-hourly cycle in different groups of animals (six mice per group).

After injection of the test dose of diazepam the mice quickly (after a latent period of 1-3 min) assumed the side position. The individual duration of pharmacologic sleep varied appreciably within each group. However, analysis of the mean values of this parameter indicated the presence of a distinct circadian rhythm. The duration of sleep reached a maximum in the middle of the light period of the day (2 p.m.). The total time of diurnal sleep was much greater than nocturnal (Fig. 1a). This is in agreement with the ethologic characteristics of mice, which lead a nocturnal mode of life, and it emphasizes once again the close similarity between the response to benzodiazepines and the physiological state of animals.

After preliminary injection of propranolol, which itself has no sedative properties, the circadian rhythm of the duration of sleep changed appreciably. Indeed, it could be said to be completely inverted, for the longest hypnogenic effect was now observed during darkness, and during light it was shortened. Two acrophases of the duration of sleep were found at night, namely at midnight and 4 a.m. Differences between the diurnal and nocturnal values in the control and experimental series were statistically significant (Fig. 1a).

The nature of this clear inversion of the circadian curve of activity of the benzodiazepine tranquilizer propranolol is not yet clear. However, it will be noted that evident antagonistic relations exist between diazepam and beta-adrenoblockers with respect to EEG data obtained on cats also [3].

The curve of the efficacy of hexobarbital during the 24-h period, and its relations with propranolol, appeared differently. The duration of barbiturate sleep exhibited two peaks: in the middle of the light and dark phases of the 24-h period, although the total duration of the action of the substance at night was greater, in agreement with the results of evaluation of the circadian rhythm of sleep induced by other barbiturates, notably pentobarbital [10]. The latent period of the response to hexobarbital varied substantially (from 2-3 to 15-20 min), and its value in most cases was inversely proportional to the duration of the effect.

Propranolol more often weakened the hypnogenic action of hexobarbital (Fig. 1b). The peaks of activity of the drug in the two phases of the 24-h period, typical of the control group, were reduced in amplitude. Admittedly, it was only during daylight (2 p.m.) and during the transition from light to darkness (8 p.m.) that these changes became statistically significant.

Thus under the influence of propranolol the circadian rhythm of pharmacogenic sleep induced by diazepam and hexobarbital was altered. Contrary to expectation, the changes observed probably cannot be attributed to a change in function of the pineal gland as a result of disturbance of beta-adrenergic control over its activity. Different beta-adrenoblockers are known to restrict pineal activity and, primarily, melatonin secretion [12]. Meanwhile, according to our previous observations [1], melatonin did not lengthen but, like propranolol, it shortened the duration of hexobarbital sleep in mice during the late hours of daylight, and weakened the action of the barbiturate slightly in the middle of the dark phase of the 24-h period.

The results most probably reflect involvement of propranolol in the intrinsic cerebral mechanisms of sleep regulation. The probability of a disturbance of these mechanisms is indicated by increased frequency of nocturnal awakenings and shortening of the phase of paradoxical sleep in man under the influence of various beta-blockers [7], and also the smoothing out of circadian fluctuations of the EEG and the cAMP level in the rat brain [4].

One problem remains, namely the extent to which disturbances of pharmacogenic sleep are due to the specific activity of propranolol. The grounds exist for such a view, since noradrenergic mechanisms are involved in the regulation of natural sleep processes [2]. However, according to some observations, the central properties of beta-adrenoblockers, including their effect on circadian rhythms of motor activity of animals, are nonspecific in character and are unconnected with a disturbance of beta-adrenergic transmission in the brain [6]. Our data also are against an important role of pineal adrenergic mechanisms. It is highly possible that the decisive contribution to the changes we found is made by a propranolol-induced change in function of the serotoninergic and/or the dopaminergic systems of the brain. Both are equally concerned in both the regulation of sleep and the effect of beta-adrenoblockers [6, 11].

REFERENCES

1. É. B. Arushanyan and P. Botvev Orkhii, Farmakol. Toksikol., No. 5, 10 (1991).
2. N. A. Vlasov, A. M. Vein, and Yu. A. Aleksandrovskii, Regulation of Sleep [in Russian], Moscow (1983).
3. A. A. Snisarenko, Fiziol. Zh. SSSR, No. 12, 1780 (1981).
4. T. A. Betts and C. Alford, Eur. J. Clin. Pharmacol., **28**, 65 (1985).
5. B. Lemmer, Ann. Rev. Chronopharmacol., **2**, 199 (1986).
6. B. Lemmer and G. Neumann, Arzneimittel-Forsch., **37**, 321 (1987).
7. D. Oksenberg and S. Peroutka, Biochem. Pharmacol., **37**, 3429 (1988).
8. E. Perez, G. Zamboni, R. Amici, et al., Boll. Soc. Ital. Biol. Sper., **65**, 41 (1989).
9. A. Reinberg, Psychopharmacology of Sleep, New York (1981), pp. 73-93.
10. L. E. Scheving, F. Donald, D. F. Vedral, et al., Anat. Rec., **160**, 741 (1968).
11. N. Tuross and R. Patrick, J. Pharmacol. Exp. Ther., **237**, 739 (1986).
12. M. Zatz, The Pineal Gland, Boca Raton, Florida (1981), pp. 229-242.