7. P. Greengard, Nature, 260, 101 (1976).
8. P. Greengard, D. A. McAfee, and J. W. Kebabean, Advances in Cyclic Nucleotide Research, P. Greengard et al. (eds.), New York (1978), p. 337.
9. H. U. Haring, M. Kasuga, and C. R. Kahn, Biochem. Biophys. Res. Commun., 108, 1538 (1982).
10. R. L. Huganir and P. Greengard, Proc. Natl. Acad. Sci., USA, 80, 1130 (1983).
11. M. Kasuga, Y. Zick, D. L. Brithe, et al., Nature, 298, 667 (1982).
12. R. Kramer, C. Schatter, and M. P. Zahler, Biochem. Biophys. Acta, 288, 146 (1972).

# COMBINED ACTION OF MELATONIN AND IMIPRAMINE ON THE STRUCTURE OF FORCED SWIMMING AND THE CIRCADIAN RHYTHM

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Mental depression is accompanied by disturbances of activity of the pineal gland. In particular, the character of secretion of its principal hormone, melatonin, is modified [4, 9]. Meanwhile, various antidepressants have been shown to interfere with melatonin synthesis [5, 10]. However, the connection between these changes and the specific activity of the drugs remains open.

The aim of this investigation was to study the character of interaction of the effects of imipramine, an antidepressant, and of exogenously administered melatonin. The forced swimming test was used as a favorite model for evaluation of antidepressant activity [6, 8]. The combined effect of the substances on the circadian rhythm of mobility, the organization of which depends on participation of the pineal gland [3], also was investigated.

## EXPERIMENTAL METHOD

The character of forced swimming (FS) was studied in experiments on 40 male mice weighing 25-30 g. The animals were kept for 6 min in a cylindrical glass vessel filled with water (28-29°C). Movements of the mice were recorded by means of an ink writer throughout the experiment. The method used enables the time course of the components of FS to be assessed. Consideration was paid to the average (in 1 min) duration of periods of immobilization, its latent period (until appearance of the first episode of immobilization), the duration of active (intensive swimming movements) and passive (low amplitude of movements) swimming, the number of transitions from one state to another, and also the number of attempts to get out of the vessel.

The animals as a whole were divided into four equal groups: 1) control (injection of 0.14 M NaCl); 2) animals receiving melatonin (synthesized at the All-Union Pharmaceutical Chemical Research Institute, Moscow, under the direction of Professor N. N. Suvorov) 1 mg/kg, intraperitoneally 30 min before testing; 3) mice receiving imipramine (10 mg/kg) daily for 2 weeks; 4) animals receiving melatonin against the background of chronic administration of the antidepressant (on the day after the last injection). The animals were kept in natural daylight and under standard conditions (food, ambient temperature) and the experiments were carried out during the afternoon (1-3 p.m.).

The circadian rhythm was evaluated in 24 rats of both sexes, weighing 150-180 g. To study the circadian rhythm of motor activity, an actograph of our own design was used. It

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enabled the number of excursions in the individual living cage to be recorded continuously for many days. Each excursion was recorded on paper tape, and as a result an actogram for a period of several days was obtained. On the basis of this actogram a chronogram was constructed (by counting the number of excursions during every 3 h). To begin with the initial dynamics of circadian activity was determined over a period of several days. Eight rats were then given melatonin (10 mg/kg, between noon and 3 p.m.). The remaining animals received imipramine (10 mg/kg) for 2 weeks, after which the effect of melatonin was tested once. The rats were maintained on a fixed schedule of light (12 h) and darkness (12 h) and had free acess to water and food.

The results were subjected to statistical analysis by the Student and Wilcoxon–Mann–Whitney tests [2].

## EXPERIMENTAL METHOD

According to previous data [1] melatonin, in the dose tested, causes distinct inhibition of spontaneous locomotion of rats. Although in the present study the same trend of its effect was observed, no gross disturbances in the character of FS could be found. In particular, melatonin limited to some degree the duration of active swimming by the mice but had virtually no effect on the number of attempts to escape from the vessel. Meanwhile, under its influence, the duration of the state of immobilization was quite clearly increased and the latent period of appearance of the first immobilization was considerably shortened. The effect of melatonin as reflected in this parameter could be regarded as a depression-inducing action, as the degree of weakening of immobilization is taken as the criterion of antidepressive activity [8]. Meanwhile, the character of the rhythmic structure of FS changed: Under the influence of the substance the animals switched from active swimming to immobility and back again more often than in the control group.

Chronic administration of imipramine, in complete agreement with observations of other workers, is accompanied by an increase in mobility of the mice, as could be judged by the tendency for the number of active attempts to escape to increase, immobilization to be shortened, and its latent period lengthened. Short-amplitude cycles were recorded more often in the time course of FS due to the easier switch from active swimming to passive.

When melatonin was administered against the background of the antidepressant, synergism was found in the action of the drugs. Evidence of relations of this kind was given by the increase in the number of attempts to escape from the vessel and the marked potentiation of the anti-immobilization effect of imipramine, with simultaneous shortening of the latent period of immobilization. Changes of this kind were statistically significant. The synergism described also was discovered by analysis of the individual pattern of the actograms of FS, in the form of a sharp decrease in the number of cycles of active swimming and immobilization, due to the more frequent switches from active to passive swimming. Thus, by contrast with the depression-inducing effect of melatonin when given alone, when given in combination with imipramine the hormone definitely potentiated its antidepressive properties.

Under the influence of melatonin inhibition of the average mobility of the rats in the 24-h period was observed. Immediately after injection of the hormone, as a rule it increased, but later activity declined, especially during the night (3-6 a.m.). The trend of the shift was independent of individual differences between the animals and, in particular, of the initial level of mobility. Analysis of the averaged chronogram emphasized depression of the circadian rhythm of activity without any displacement of the acrophase of the rhythm.

A single injection of imipramine was followed by increased average mobility of the rats in the 24-h period. Moreover, toward the end of the first week of administraton of the drug this effect weakened, and after another week there was a tendency for total motor activity in the group of animals as a whole to decline. A clear shift of the acrophase of the rhythm to late times was found in such cases on the averaged chronogram (3-6 a.m. instead of 9 p.m. to midnight in the control). As the results of these experiments, and with another antidepressant, amitriptyline (data not given), showed, such a shift is very typical of drugs of this kind.

The use of melatonin against this background modified the action of imipramine in a definite manner. The amplitude of the circadian waves of activity decreased even more intensively, but the acrophase was shifted to earlier times (between 9 p.m. and midnight).

According to the preliminary data, the effectiveness of different tricyclic antidepressants relative to the circadian rhythm of motor activity of rats is largely dependent on individual differences between the animals. On this basis, it was decided to separate the rats of the group under study into those with initially high and low mobility (four rats in each subgroup) and to evaluate their sensitivity both to imipramine alone and to its combination with melatonin, separately.

Rats with a high level of activity proved to be more resistant to the antidepressant. This was shown, on the one hand, by the weaker inhibitory effect of the drug on locomotion and, on the other hand, the well marked features of tolerance, if judged by the character of migration of the acrophase of the rhythm. Whereas after the first week of administration of the drug it shifted toward later times, after 2 weeks there was a shift of the acrophase in the opposite direction. In the animals of this group melatonin depressed circadian activity more strongly and, like the antidepressants, shifted the acrophase of the rhythm toward the early morning hours. In other words, in that situation the hormone behaved like an imipramine agonist.

The effect of the drugs appeared different in rats with initially low values of mean 24-hourly mobility. They responded much more strongly to the depriming action of the antidepressant, and after its administration for 2 weeks the acrophase of the circadian rhythm remained steadfastly shifted toward later times. Under these conditions melatonin behaved like an imipramine antagonist, increasing the amplitude of the circadian rhythm and normalizing the position of its acrophase.

Thus, whereas the combined approach to evaluation of interaction between imipramine and melatonin, on the model of the circadian rhythm of motor activity, indicates that the character of their relations is antagonistic, by analysis of the pharmacologic sensitivity of individual groups of animals it is revealed that the hormone has a modulating, adaptive influence on the effect of the antidepressant.

The distinct synergism in the action of imipramine and melatonin on individual parameters of FS, discovered in experiments on mice, could be logically explained by pharmacokinetic interaction between the two substances. At least we know that melatonin can modify the activity of certain microsomal enzymes of the liver [7]. However, both the results of the experiments on rats described above and our observations with repeated use of melatonin against the background of the antidepressant, point rather to an important role of the pharmacodynamic factor in these interrelations. In particular, the modulating effect of melatonin on the function of brain receptors, participating in the effect of antidepressants, cannot be ruled out. Evidence of the possibility of such a shift is given by data showing that the pineal hormone, in lower doses, changes the state of central dopaminergic mechanisms in opposite directions, depending on their initial state [1].

## LITERATURE CITED


1. É. B. Arushanyan and K. B. Ovanesov, Farmakol. Toksikol. (1988) (in press).
2. E. V. Gubler, Computerized Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
3. E. I. Chazov and V. A. Isachenkov, The Pineal Gland: Its Place and Role in the System of Neuroendocrine Regulation [in Russian], Moscow (1974).
4. B. Claustrat, G. Chazot, J. Brun, et al., Biol. Psychiatr., 19, No. 8, 1215 (1984).
5. P. J. Cowen, S. Fraser, D. C. Grahame-Smith, et al., Br. J. Pharmacol., 78, No. 1, 89 (1983).
6. S. K. Kulkarni and M. P. Parale, Meth. Find. Exp. Clin. Pharmacol., 8, No. 12, 741 (1986).
7. V. Marks, J. English, W. Akerne, et al., Clin. Biochem., 18, No. 1, 154 (1985).
8. R. D. Porsolt, A. Berlin, and M. Jalfre, Arch. Int. Pharmacodyn., 229, No. 2, 327 (1977).
9. L. Wetterberg, Psychoneuroendocrinology, Vol. 8, No. 1, 75 (1983).
10. A. Wirz-Justice and J. Arendt, Lancet, 1, 425 (1980).