EFFECT OF MELATONIN AND PINEALECTOMY ON BRAIN ELECTRICAL ACTIVITY OF RATS AT DIFFERENT TIMES OF DAY

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The principal pineal hormone melatonin has a varied effect on brain activity [1]. Meanwhile, the natural secretion of melatonin by the gland in animals of different species exhibits a distinct circadian rhythm with a peak during the night [11], and this may account for variations in the level of the psychotropic action of the hormone during the 24-h period [3].

The aim of this investigation was to detect changes in brain electrical activity following administration of various doses of melatonin, and in animals with pineal deficiency due to removal of the gland, at different phases of the circadian rhythm. Special attention was paid to intermediate times between nocturnal awakening of rats and daytime rest, i.e., times when an active adjustment of pineal function is taking place.

EXPERIMENTAL METHOD

Experiments were carried out on 53 noninbred male albino rats weighing 140-200 g during the summer (June, July) months. The animals were kept under natural lighting conditions, and on a standard diet and temperature schedule. All operations were performed under pentobarbital anesthesia. To record the EEG electrodes were implanted in the sensomotor cortex and dorsal hippocampus, by the method described previously [5]. Pinealectomy was performed by a surgical method, modified in the writers' laboratory [6]. Brain electrical activity was recorded on a "Neirograf-18" electroencephalograph. Fourier spectral analysis of the EEG was carried out by means of a BAS 161 computer analyzer (from "O.T.E. Biomedica," Italy). Altogether five series of experiments were conducted on intact and pinealectomized rats, and also on rats receiving one of three doses of melatonin. The rats of each series were subdivided into two subgroups (five or six animals in each group) to evaluate the EEG parameters in the morning (6-9 a.m.) and evening (6-9 p.m.). Melatonin (0.1, 1, and 10 mg/kg, intraperitoneally) was given in a single dose, as was also the case with injection of physiological saline in the control group, 30 min before the EEG was recorded. The effects of pinealectomy were assessed 10 days after removal of the gland Animals undergoing a mock operation served as the control. The results were subjected to statistical analysis by the ANOVAR method for simultaneous analysis of variations in mean values of several series of samples [8]. To assess tied pairs, Student's test and the Wilcoxon–Mann–Whitney test were used.

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EXPERIMENTAL RESULTS

Although the EEG parameters of brain activity on man and animals are known to exhibit circadian oscillations [12], on visual assessment of cortical and hippocampal electrical activity during the morning and evening, no differences in the EEG pattern could be detected. Admittedly, as the results of spectral analysis show, in both these brain structures the power of oscillations with a frequency of 8-10 Hz, and also in the high-frequency region of the spectrum (over 16 Hz) was higher at the end of the daylight phase of the 24-h period than in the morning.

This fact is in agreement with observations showing that the energy of the high-frequency component of the EEG reaches a maximum in rats at the beginning of the period of darkness [13], and may be evidence of a raised level of consciousness.

Considering that natural melatonin secretion in the pineal gland increases with the transition to darkness, the effects of the exogenous hormone during the evening are particularly interesting As the experiment showed, melatonin induced dose-dependent (0.1, 1, and 10 mg/kg) modification of the EEG, as shown by weakening of its power throughout the spectrum both in the neocortex and in the hippocampus. Polymorphic discharges were replaced by a monotonous rhythm. As a result, against this background although the spindle potentials did not disappear, they were much more difficult to distinguish (Fig. 1a, II). The marked variations of individual sensitivity of the animals to melatonin, which were particularly significant after injection of 10 mg/kg of the substance, must also be noted. Besides cases of considerable flattening of the EEG and of low-voltage theta-activity, a regular rhythm (12-14 Hz) of moderate amplitude (about 100 μV) also appeared.

Spectral analysis, on the one hand, confirmed the trend of the above-mentioned changes, and on the other hand, revealed the most significant changes in the frequency pattern of the EEG. A smaller dose (0.1 mg/kg) of melatonin led to limitation of the theta-rhythm, namely oscillations within the 4-8 Hz band. A clear decrease of beta-activity also was observed in the high-frequency region of the spectrum. With an increase in the dose, after 1 and, in particular, 10 mg/kg melatonin, depression of the slow rhythm became somewhat weaker, although its power still remained below the control values, whereas weakening of the power of oscillations with a frequency of 6-12 and 12-22 Hz continued to progress. On the whole, compared with the control animals, reduction of the fraction of waves within these bands following administration of 10 mg/kg of melatonin was statistically significant (Fig. 2a). Comparison of changes in electrical activity of the sensomotor cortex and hippocampus under the influence of melatonin suggests that the hormone interferes more strongly with the function of the latter. This is also shown by the more marked limitation of the power of the segments of the EEG spectra, observed above, in the rat hippocampus.

Whereas in the evening melatonin induced a significant decrease in spectral density of the EEG parameters, when they were recorded at the beginning of the daylight period, no such effect was present. There was rather a tendency toward strengthening of oscillation in the region of both low and high frequencies, but the change was not statistically significant.

The EEG recorded in the cortex and hippocampus of pinealectomized animals was outwardly not significantly different from that of the intact rats. There were no marked differences either at different times of the 24-h period, Electrical activity as a whole remained polymorphic, by contrast with the monotonization typical of melatonin. The only sign drawing attention to itself, according to our observations, was the appearance of more clearly defined spindles in the EEG pattern (Fig. 1b, II).

Meanwhile, according to the results of spectral analysis, pinealectomy nevertheless induced definite changes in the rhythmic structure of the EEG, more especially during the morning. Compared with intact animals, the power of the oscillations was greater in the middle (8-12 Hz) and high-frequency (14-22 Hz) bands. In the latter case, the shift was significant in character (Fig. 2b).

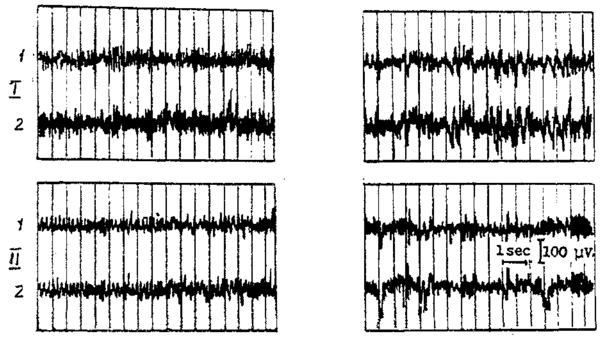

Fig. 1

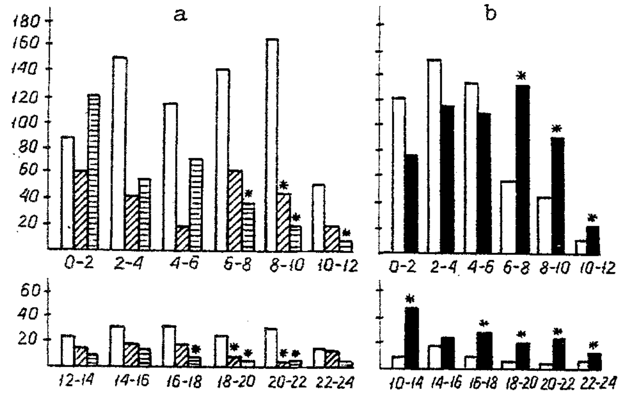

Fig. 2

Fig. 1. Effect of melatonin (evening, a) and of pinealectomy (morning, b) on spontaneous EEG of rat sensomotor cortex (1) and hippocampus (2). I) Control animals; II: a) after injection of 10 mg/kg melatonin; b) pinealectomized animal.

Fig 2. Changes in spectral characteristics of EEG of groups of rats receiving melatonin (a) and undergoing pinealectomy (b). Unshaded columns – effect on control animals, obliquely shaded – after 0.1 mg/kg, and horizontal shading – after 10 mg/kg melatonin; black columns – after pinealectomy Vertical axis – power of EEG spectrum (in μV); horizontal axis – frequency range (Hz). Asterisk indicates statistically significant ($p < 0.05$) differences compared with control values.

Thus melatonin and pinealectomy, while not causing gross disturbances in the pattern of the spontaneous EEG, nevertheless lead to changes in the spectral density of its rhythmic components. Evidence of the specificity of these changes is given by the mirror-image nature of its effects: under the influence of melatonin significant changes arose in the evening, but after pinealectomy in the morning. These two procedures had opposite effects on the abundance of high-frequency oscillations. This type of circadian rhythm is in agreement with results obtained on other models in which melatonin was given or the pineal gland removed [2, 10]. The probable cause is the fact that at the end of the daylight phase of the 24-h period, before the rise of the level of natural hormone secretion, the sensitivity (density) of free melatonin receptors was high, but in the morning, opposite relations hold good [14].

The character of reorganization of the EEG spectrum in response to a change in pineal function deserves special attention. The established tendency toward limitation of the theta-rhythm, which is regarded as an indicator of an anxiolytic effect [4], may reflect the known tranquilizing properties of melatonin and its ability to interact with GABA-ergic brain mechanisms [7, 9]. On the other hand, depression of beta-activity by melatonin can be regarded as a unique equivalent of its hypnogenic action [9], whereas the morning potentiation of high-frequency rhythmic activity after pinealectomy evidently serves as criterion of a rise of the level of wakefulness of the animals.

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