EFFECT OF PINEALECTOMY ON THE HYPNOGENIC ACTION OF HEXOBARBITAL IN RATS

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The pineal gland in the brain is involved in the maintenance of the circadian rhythm [5, 7]. The principal pineal hormone, melatonin, in man and different species of animals, is actively secreted during the period of darkness, so that it has been classed as one of the natural sleep regulators [8]. In that case, various procedures aimed at and activities performed by the pineal gland ought, on a priori grounds, to be reflected in the hypnogenic activity of drugs. In order to test this hypothesis, in the investigation described below the features of the hypnogenic action of a barbiturate were studied after removal of the pineal gland and administration of melatonin.

EXPERIMENTAL METHOD

Altogether, three groups of experiments were carried out (seven animals in each group) on noninbred male rats weighing 140-200 g, during the winter (January-February). The experiments of group 1 were performed on intact rats, those of group 2 on rats undergoing a mock operation, and those of group 3 on pinealectomized rats. The operation was performed under pentobarbital anesthesia (40 mg/kg, injected intraperitoneally like all other drugs). The pineal gland was removed by a technique developed in our laboratory [4]. For the mock operation, the parietal bones were trephined and the dura opened anteriorly to the projection of the pineal gland on the skull. The experiments began on the 50th day after the operation.

In all experiments the rate of onset and duration of pharmacologic sleep (as shown by the length of time spent by the animal in the side position) were assessed after injection of a standard dose (60 mg/kg) of hexobarbital. The effect of the drug was determined twice (with an interval of 5-7 days) during daylight and darkness (1-3 a.m.) and also at the corresponding time of day or night after administration of hexobarbital preceded (15 min beforehand) by melatonin, in a dose of 0.1 mg/kg.

The animals were kept under standard conditions (number in the cage, diet, ambient temperature) with a fixed program of daylight and darkness (daylight for 12 h from 8 a.m. to 8 p.m., darkness for 12 h from 8 p.m. to 8 a.m.). The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney test [3].

EXPERIMENTAL RESULTS

In the dose used, hexobarbital induced a brief hypnogenic effect in intact animals. According to the day and night determinations it was similar in duration and latent period. Admittedly, during the dark period and the absolute values of the duration of sleep were a little smaller (8.4 min compared with 10.1 min in daylight), and the number of animals in the group which did not sleep was twice as great. This fact probably depends on etiologic features of the rats, which lead a nocturnal mode of life.

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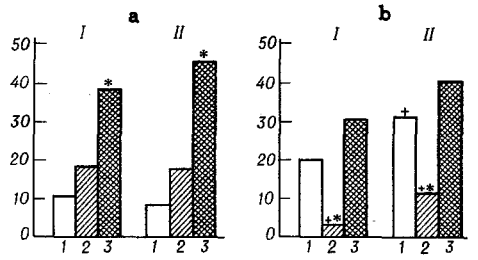

Fig. 1. Effect of pinealectomy and melatonin on hypnogenic effect of hexobarbital at different times of the 24-hour period. a) Without melatonin, b) with injection of melatonin in (0.1 mg/kg). I) Daylight, II) night. 1) Intact rats, 2) mock operation, 3) pinealectomized rats. Ordinate, duration of hexobarbital sleep (in min). Asterisk indicates statistically significant ($p < 0.05$) shift compared with parameters in intact rats; plus sign indicates significant differences compared with corresponding group of animals before injection of melatonin.

After pinealectomy a marked increase (almost fourfold) was observed in the strength of action of hexobarbital and its latent period was shortened. The same result was obtained during daylight and in darkness (Fig. 1a). The duration of nocturnal sleep, incidentally, was greater on average than that of diurnal sleep, although differences between the values determined at different times of the 24-hour period were not statistically significant. This state of affairs was confirmed by experiments with a reversed lighting schedule, emphasizing the endogenous nature of the difference discovered.

In a special series of experiments, all the rats were kept for 5 days for 12 h in darkness during the daytime, but with illumination during the night. According to preliminary observations (with V. A. Baturin), this period was long enough to reverse the pattern of mobility of the rats, to reach a maximum during the previous daylight hours. In this situation also, the action of hexobarbital lasted long on the pinealectomized animals during the period of natural night than under conditions of artificial darkness.

The results (Fig. 1a) show that the rats undergoing a mock operation also were characterized by some degree of strengthening (although not significant compared with the control) of the hypnogenic effect on the barbiturate. According to this parameter, the animals of this group occupied an intermediate position between the intact and pinealectomized rats. A similar state of affairs was previously found by other investigators, when different types of mock operation were used, although in every case it involved disturbances of the integrity of the dura mater [1, 2, 6]. Operations on the skull, impairing the normal flow and pressure of the cerebrospinal fluid evidently lead somehow or other to functional insufficiency of the pineal gland and any reference to a "mock operation" must therefore be made very conventionally.

Prolongation of the action of hexobarbital after pinealectomy can be taken as evidence that the gland produces certain biologically active substances which, under normal conditions, prevent the development of pharmacologic sleep. Moreover, these properties are evidently exhibited more strongly at night. To test this hypothesis, a series of experiments was carried out in which hexobarbital was given after injection of the pineal hormone melatonin.

In the present investigation, a relatively small dose of the hormone (0.1 mg/kg) was given, one which is used more frequently than others in experimental practice. A combination of the drugs in intact rats prolonged the hypnogenic effect of hexobarbital, and this change at night was statistically significant. However, against the background of pinealectomy, melatonin, conversely, weakened pharmacologic sleep: it limited its duration and lengthened its latent period (Fig. 1b). The antagonistic action of the hormone by day, when pineal disturbances are less marked, was exhibited more strongly. A similar result was obtained also in animals kept on a reversed lighting schedule.

Meanwhile, results at first sight paradoxical were obtained in the group of rats undergoing the mock operation. In them, melatonin limited the action of hexobarbital with a high degree of significance (compared with data for intact animals and the results of the previous series without injection of the hormone). The group of animals undergoing the mock operation differed appreciably from the other two groups in sensitivity to melatonin. Perhaps during the mock operation, under the conditions of functional insufficiency of the pineal gland which we postulated above, the antihypno-

genic properties of the hormone may be revealed much more easily than in the presence of a permanent organic defect after removal of the gland (and weakening of activity of the melatonin receptors?).

Consequently, results of this series of experiments also confirm in principle the existence of antihypnogenic properties among the pineal compounds. With what can they be connected? A change in the action of hexobarbital following different kinds of procedures interfering with pineal function, is probably due to both pharmacokinetic and pharmacodynamic mechanisms. According to our preliminary observations (with A. V. Popov and V. V. Zarubin), pinealectomy is accompanied by weakening, and injection of melatonin by strengthening of the activity of certain microsomal enzymes in the rat liver. Modification of the processes of hexobarbital biotransformation is therefore quite possible. On the other hand, the possibility cannot be ruled out that in animals with a diurnal and nocturnal mode of life, melatonin plays a different biological role. Although maximal secretion of the hormone for both types was shown to be in the dark period of the 24 hours, nocturnal animals exhibit increased behavioral activity at this period, unlike diurnal animals. Hence, it follows that whereas in man, for example, melatonin ought to be prohypnogenic, in rats it is more likely to be an antihypnogenic factor.

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