DELAY OF AMPHETAMINE TOLERANCE IN RATS AFTER PINEALECTOMY

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KEY WORDS: amphetamine; tolerance; rat pineal gland

Melatonin, the principal pineal hormone, has a modulating effect on dopaminergic transmission in the brain, judging by the change in dopamine secretion processes in different parts of hypothalamus and hippocampus [10]. Other supporting evidence is given by the reorganization of the time course of apomorphine stereotypy under the influence of melatonin and pinealectomy [1, 2].

Taking this, and also ideas on the adaptive properties of the gland [6], into consideration, we have studied the character of formation of tolerance to the dopamine agonist amphetamine, on a model of amphetamine stereotype in rats. Minute by minute fluctuations in the pharmacologic response were evaluated as a more sensitive indicator of drug resistance [3].

EXPERIMENTAL METHOD

Experiments were carried out on 28 male Fisher albino rats weighing 180-240 g. Monotonous head movements induced in the animals by amphetamine were used as the indicator of stereotypy. Automatic movements were recorded in a special soundproofed chamber (measuring 40 × 30 × 30 cm), by means of a flexible electrically conducting collar which the animal wore around its neck. By this means, regular action potentials of the neck muscles, corresponding to head turning, were recorded on the tape of an electroencephalograph. Primary processing of the traces thus obtained consisted of counting the number of these movements each minute, and using the data to construct chronograms. Analysis of these records enabled the mean intensity of stereotypy throughout the experiment and the amplitude-frequency characteristics of the process to be calculated, including by the use of spectral analysis of the curves.

Effects of acute and chronic (for 14 days) administration of amphetamine in a standard dose (5 mg/kg, intraperitoneally) were analyzed. The behavioral changes were recorded immediately after injection of the drug and at the same time of day. These determinations were made on animals of three groups: intact; pinealectomized, and undergoing a mock operation (trephining of the skull anteriorly to the coronal suture). The technique of pinealectomy was a version of that in [5], modified in our laboratory. The animals were used in the experiments 2 weeks after the operation. They were kept under conditions of standard temperature and alternation of light and darkness, and had free access to food and water. The results were subjected to statistical analysis by Student, Wilcoxon, and Wilcoxon–Mann–Whitney tests [4]. Calculations were done on the "Élektronika BK-0010-01] computer.

EXPERIMENTAL RESULTS

Stereotyped behavior by the rats lasted 2-3 h after a single injection of amphetamine. Continuous electromyographic recording of this state demonstrated its unsteady, fluctuating character. Minute waves with different periods (from 2-3 to 16 min or more) and a phasic structure of the process (formation, stabilization, and decay of steroeotype) could be distinguished on the trace of the primary chronogram.

The effect of amphetamine was stonger in the pinelacetomized rats than in intact animals. This was shown by the more marked increase in the frequency of automatic movements during the first 10-30 min of the chronogram. The mean intensity of stereotypy, determined as the number of head movements per minute, also was found to be higher, for the group of animals as a whole (79.0 ± 7.5 after pinealectomy, 54.6 ± 3.2 movements/min in the control). Both the initial phase of the process (36.4 ± 6.2 compared with 24.4 ± 2.5 min in intact

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Department of Pharmacology, Stavropol' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. A. Kharkevich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 42-44, January, 1990. Original article submitted April 3, 1989.

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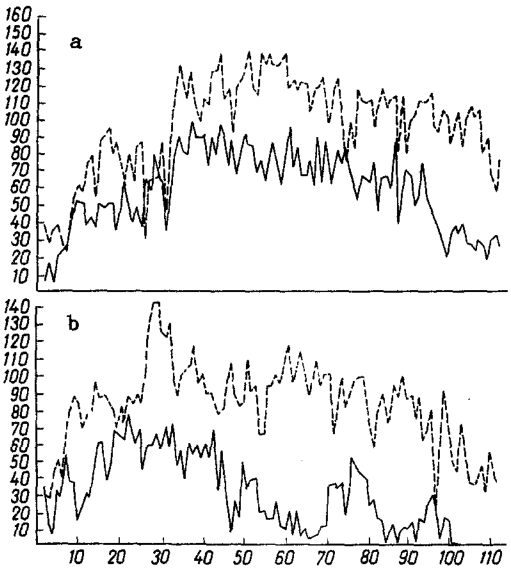

Fig. 1

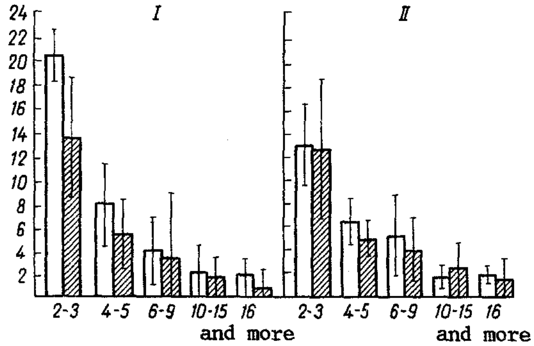

Fig. 2

Fig. 1. Effect of pinealectomy on time course of stereotypes behavior in individual rats during acute (a) and chronic (b) administration of amphetamine. Curve shows minute by minute course of frequency of stereotypes head turning by the animal in the control (continuous line) and after pinealectomy (broken line). Abscisa, duration of recording (in min); ordinate, absolute number of head movements per minute.

Fig. 2. Histogram of stereotypes behavior of intact (I) and pinealectomized (II) rate. Columns show number of waves of a particular waveband on chronograms (unshaded – acute, shaded – chronic injections of 5 mg/kg of amphetamine). Abscissa, period of waves (in min); ordinate, number of waves.

rats) and the period of its stabilization (77.9 ± 8.1 compared with 60.3 ± 4.8 min in the control) were significantly lengthened. Histogram analysis of the fluctuating curve revealed some decrease in amplitude of the shortest waves (2-3 min) after pinealectomy (Figs. 1 and 2). As regards the basic parameters of stereotypy, and whether amphetamine was administered acutely or chronically, rats undergoing the mock operation occupied an intermediate position between the pinealectomized and intact rats.

Our preliminary study of the character of the time course of stereotypy during repeated injections of amphetamine indicated differences in the evolution of the fluctuating curve on account of the unequal sensitivity of individual rats to the drug. By this criterion, three categories of animals could be conventionally distinguished: the first responded to a 2-week course of amphetamine by strengthening of the automatic movements, the second responded by weakening, and the third category showed no significant effect. In principle it is important to note that, the higher the initial response to acute administration of amphetamine and the greater the proportion of oscillations with a short period in its structure, the stronger the tolerance. In other words, from the chronobiological standpoint, the factor determining the subsequent formation of resistance to amphetamine is the presence of a well marked oscillatory process consisting of waves in the minute band, which is a unique indicator of the high adaptive capacity of the brain.

Tolerance developed with much greater difficulty after pinealectomy, and the relationship indicated above was less distinct in character. The integral chronogram for the group of animals in the control differed from that of the pinealectomized rats in its smoother rise, its lower amplitude, and its shorter duration. The same rule also was found when individual chronograms were compared (Fig. 1b).

This conclusion also was confirmed by a closer analysis which took into account the differences in evolution of stereotypy during chronic amphetamine administration in individual groups of animals. For this purpose we compared the rate of formation of drug resistance in those intact and pinealectomized rats which initially had given the strongest response to the drug (there were four of them in each group). In intact animals of this kind, subsequent weakening of stereotypy was very demonstrative and the mean (for the experiment) frequency of automatic movements fell by 35.5% (from 69.2 ± 1.6 to 46.7 ± 6.2 movements/min). Meanwhile, in the subgroup of pinealectomized animals, with similar characteristics, the fall in the mean value of this parameter was much less – by only 0.7% (from 95.1 ± 1.8 to 86.8 1 7.1 movements/ min).

In some control animals, with initially less marked behavioral disturbances, after chronic administrative of amphetamine signs of intensification of its pharmacologic effect were found (it increased on average for five rats from 56.2 ± 1.7 to 66.1 ± 3.0 movements/min, i.e., by 17.6%). However, for the subgroup of pinealectomized rats with similar sensitivity to amphetamine, this change was much greater (90.1 ± 1.2 compared with 58.4 ± 0.3 movements/ min, an increase of 54.3%).

Yet another sign of the higher sensitivity of pinealectomized rats to amphetamine was the modest reorganization of the time course of stereotypy. Tolerant rats in the control showed a decrease in power of the oscillatory process with a marked limitation of the value of all harmonics studied. The most sensitive indicator in this case was a decrease in the number of waves with a short period (2-3 min). In the analogous population of pinealectomized rats, their number remained at the same level (Fig. 2).

Thus to judge from the differences in the time course of stereotypes behavior, the effect of acute administration of amphetamine is manifested more strongly after preliminary pinealectomy, and the formation of tolerance to it is more difficult. There are two possible explanations of this.

First, it is logical to look for the cause in differences in the action of pineal factors on activity of brain dopoaminergic structures. According to recent data, evolution of behavioral responses to chronic administration of amphetamine depends on a change in the number and density of dopamine receptors or on the level of secretion and accumulation of the mediator [7-9]. Meanwhile melatonin, to which the basic functional properties of the pineal gland are ascribed, has a definite involvement in these processes, on which it most frequently has a depressant effect [2, 10].

The other alternative, but quite likely explanation is that pinealectomy causes certain disturbances in the biotransformation of amphetamine and thereby delays tolerance formation. This hypothesis is supported by the results of our previous experiments (conducted with V. V. Zarubin), showing that pinealectomy is accompanied by reduced activity of several microsomal enzymes in the rat liver, notably cytochrome P-450, evidently because of prevention of the inducing effect of melatonin.

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