EFFECT OF PINEALECTOMY ON TIME COURSE OF APOMORPHINE STEREOTYPY IN RATS

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The pineal gland participates in control over several brain functions. This is shown by changes in electrical activity of the brain and certain forms of behavior of animals after pinealectomy (PE) [5, 6]. Meanwhile, the principal pineal hormone, melatonin, has been shown to possess neurotropic properties on resorptive administration [7].

On the basis of facts such as these, in the present investigation the writers attempted to determine indirectly the role of pineal hormones in the state of central dopaminergic mechanisms. For this purpose, the time course of the action of apomorphine, which stimulates dopamine receptors, was studied in rate after PE.

METHODS

Experiments were carried out on 34 noninbred male albino rats. As a first step the time course of stereotyped behavior of all the animals was determined after intraperitoneal injection of a standard dose (1 mg/kg) of apomorphine. Stereotypy was recorded visually every 5 min until its complete disappearance, and the number of monotonous head movements during 1 min was counted. It was shown previously, on a model of amphetamine stereotypy, that behavior of this kind is an unsteady oscillatory process [1]. Accordingly, at the height of action of apomorphine (from the 20th to the 35th minute) stereotypy was assessed every minute for 15 min.

The investigation was conducted in two stages — in the autumn and winter months. On the basis of information to the effect that pineal function is more important for young animals [4], the autumnal series of experiments was carried out on 18 rats of two different age groups: young (7-8 weeks, weight 80-120 g) and old (26-28 weeks, weight 250-300 g). Each group, in turn, was divided into two subgroups (with four or five animals in each subgroup). The animals of one of them served as the control, and underwent a mock operation (MO), and rats of the other subgroup underwent PE. Since particular changes in the character of apomorphine stereotypy were found only in young animals, the experiments in the winter months were repeated on eight rats of the same age, which also were divided into two subgroups (MO and PE).

PE was performed under pentobarbital anesthesia without injury to the sagittal sinus. In MO, the bone and dura were divided anteriorly to the pineal gland, so as not to disturb its innervation and blood supply. In addition, various types of operations were performed on eight animals to determine the most appropriate technique of MO. These experiments showed that wide division of the dura (as during PE) was an essential condition for an adequate control.

The effect of apomorphine was assessed 10 and 30 days after the operation. At the end of the experiments the rats were killed and in all cases preservation of the pineal gland and the state of the brain were verified. The experiments were carried out under natural lighting conditions, at a strictly definite time during daylight (3.30-5.00 p.m.) and standard conditions of feeding and maintenance of the animals were observed. The results were subjected to statistical analysis by the Student and Wilcoxon-Mann-Whitney tests [3]. Autocorrelation and spectral analysis were performed by the EC-1020 computer.

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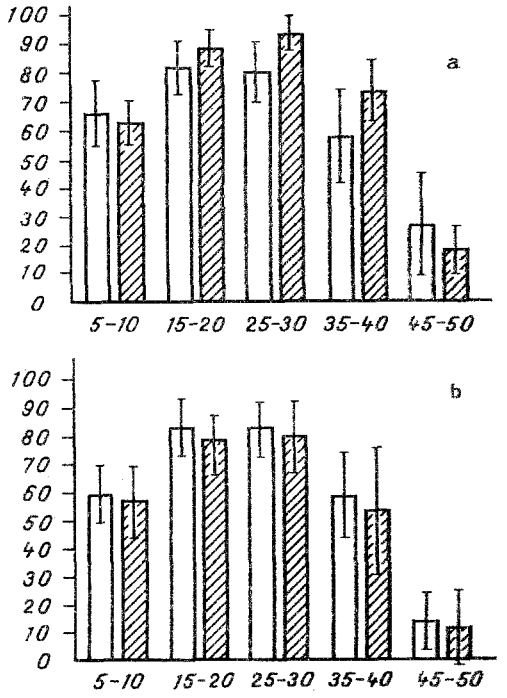

Fig. 1. Effect of PE on dynamics of frequency of stereotyped movements. Abscissa, time (in min); ordinate, absolute number of head movements. Columns show number of automatized movements after injection of a standard dose of apomorphine (1 mg/kg) before (unshaded) and 10 days after (shaded) operation. Mean results of two determinations (with interval of 5 min between them) on young animals in the winter series of experiments are shown. a) MO, b) PE.

RESULTS

The first signs of stereotypy were found quite quickly (only 15 min) after the injection of apomorphine. The number of automatized head movements reached a maximum after 20-30 min, and thereafter it fell progressively with normalization of activity after 55-60 min.

PE as a whole caused no significant change either in the animals' behavior or the intensity of apomorphine stereotypy, or the character of its time course. At the postoperative times studied after PE the rats behaved in the open field test or in a Y-maze just like the animals with MO. The mean times of the beginning of stereotypy, the times when it reached its maximum, and the times of its decline did not differ significantly from the control values. No differences likewise were found in the structure of abnormal behavior: after both PE and MO, monotonous sniffings, vertical standings, or head dippings in a holeboard appeared with about equal frequency.

However, with a more careful study of the individual responses to apomorphine, attention was drawn to one fact. Frequently in repeated experiments the response to the drug increased, to judge by the frequency of stereotyped head movements, and evidently because of sensitization of post-synaptic dopamine receptors. This phenomenon was observed only in cases when at the peak of the apomorphine effect the original frequency of turns was rather low (under 60-70 turns/min). In these cases the number of movements after 20 min of recording increased regularly (up to 80-90/min). If, however, the initial activity at the maximum of the effect was high, it did not change during repeated experiments. This tendency was equally marked in rats after PE and MO, except in young rats after PE.

Statistical analysis of the parameters of stereotypy for the animals of this category (autumn and winter groups) indicated that in young rats the tendency noted above was present

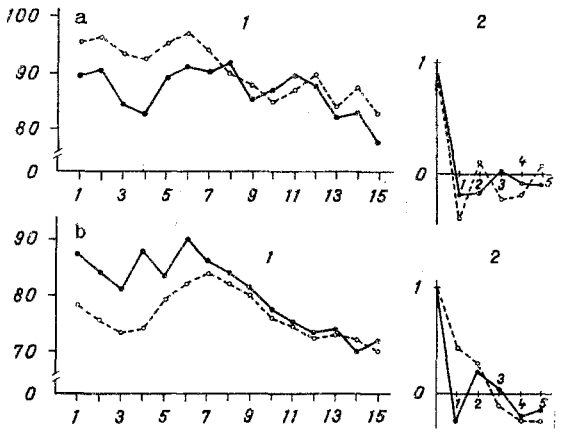

Fig. 2. Slowing of rhythmic oscillations of apomorphine stereotypy in young rats after PE (winter series of experiments). 1) Time course of number of stereotyped movements (minute by minute recording at height of action of apomorphine) for same rat before (continuous line) and 10 min after (broken line) operation; 2) results of autocorrelation analysis of the same experiments. Remainder of legend as to Fig. 1.

after MO, and the shift reached statistical significance after 25 min of recording. Meanwhile, after PE, no such tendency was observed in this same age group (Fig. 1). This state of affairs justified a more detailed study of the minute by minute dynamics of stereotyped behavior.

It was shown that apomorphine stereotypy, like that due to amphetamine, has a distinct time structure. Visually, the first time courses differed in amplitude and period of oscillation. Histogram analysis showed that the predominant waves had a period of 2-3 min, and this accounted for 60-70% of all waves. Slower fluctuations (with a period of 4-5 min) were observed less frequently (10-15%). The same ratio was found in the majority of groups irrespective of the animals' age or the season of the year. This general rule and the fluctuating nature of apomorphine stereotypy itself were confirmed by autocorrelation and spectral analysis (Fig. 2).

Meanwhile, in one of the experimental series (in young rats with PE, in the winter) reorganization of the rhythmic structure of the stereotypy was found. In the early and late stages after the operation, quite definite slowing of fluctuation was observed in these animals. In all the rate of this group without exception, besides a shift of the peak of spectral density toward lower frequencies, the decline of the self-oscillating function (SO-function) of the autocorrelograms was slowed (Fig. 2). Comparison with results obtained on animals of the same age undergoing the mock operation in the same season (four animals in each case) showed that the parameter lk, characterizing the rats of decline of the SO-function [2], was regularly higher after PE. Comparison of the mean values of lk for rats undergoing PE and MO, using the Silcoxon–Mann–Whitney nonparametric test [3] demonstrated the high significance of the differences ($P < 0.01$). Meanwhile, in old (irrespective of season) and young animals, no such shift was observed in the fall.

PE as a whole thus causes no significant changes in the rats, intensity, or duration of apomorphine stereotypy. However, a detailed analysis showed that PE on young animals prevents strengthening of motor automatisms in repeated experiments and, in the winter months, leads to slowing of minute fluctuations of abnormal behavior.

The absence of any gross changes in activity of the nigro-striatal dopaminergic mechanisms, with which the genesis of stereotypy is associated, after PE, is in agreement with data in the literature [9], according to which pineal melatonin is not involved in the release of striatal dopamine, although it modifies this process in the hypothalamus and hippocampus.

These results are evidence that the pineal gland, in a certain category of animals and at a strictly definite time of year, is evidently involved in the control of activity of

nigro-striatal mechanisms. In particular, melatonin or other pineal hormones evidently facilitate some degree of sensitization of postsynaptic dopamine receptors, to judge by the absence of increase of the response to apomorphine in repeated experiments after PE. Slowing of minute fluctuations of stereotyped movements in young rats after PE is yet another argument in support of the possible existence of such an effect.

Meanwhile this last observation may indicate a pulsating character of the release of pineal hormones, which is reflected in the rhythmic structure of stereotyped behavior. In fact, for example, distinct minute fluctuations of the melatonin level have been found in human plasma [8]. Probably for this reason, removal of the pineal gland leads to defects in the temporal organization of the dopaminergic mechanisms of the brain.

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AN ACTIVE ANALOG OF INACTIVE "SLEEP PEPTIDE"

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Delta sleep-inducing peptide (DSIP) is a nonapeptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), isolated from blood flowing from the brain of rabbits exposed to low-frequency electrical stimulation of the intralaminar thalamic nuclei [13]. Despite intensive study of "sleep peptide" from various aspects [6, 12], its principal (hypnogenic) property remains at least in doubt [1, 5, 11, 14]. Data on the effect of this peptide on temperature regulation are equally contradictory [7, 15]. However, we know that slight modifications to the structure of neuropeptides, aimed at making them more resistant to proteolysis, such as by substituting certain L-amino acid residues for their optical D-isomers, can in some cases lead to a significant increase in their biological activity [3]. The study of proteolysis of the DSIP molecule through the action of brain enzymes in vitro has shown that the primary event is removal of the N-terminal tryptophan residue [9, 10]. The D-Trp^1 analog of DSIP was accordingly synthesized and its physiological activity studied.

METHODS

This peptide was synthesized by the classical method among other members of the group of structural analogs of DSIP [4]. The dry peptide was kept in a refrigerator at between 0 and

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