EFFECT OF INJECTION OF DELTA SLEEP-INDUCING PEPTIDE INTO THE SUBSTANTIA NIGRA ON THE PARKINSONIAN SYNDROME IN RATS

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The development of various forms of parkinsonian syndrome has been shown to be based on the formation of a generator of pathologically enhanced excitation (GPEE) in the caudate nuclei [3-5]. One mechanism of the appearance of such GPEE is a fall in the level of nigrostriatal inhibitory control [3, 6], in the realization of which an important role is played by substances of peptide nature [9]. We have studied behavioral and electrographic changes in animals arising in response to injection of delta sleep-inducing peptide (DSIP) into the substantia nigra (SN) of rats.

EXPERIMENTAL METHOD

Acute and chronic experiments were carried out on 50 Wistar rats weighing 250-320 g. Under hexobarbital anesthesia (100 mg/kg), and taking coordinates from the atlas [11], guiding cannulas (external diameter 0.5 mm) were implanted into the rostral zones of the caudate nuclei (AP = 0; L = 2.5; H = 3.5), SN (AP = −5.3; L = 2.5; H = 8.0), the hippocampus (AP = −4.0; L = 2.5; H = 3.5), and also the sensomotor cortex of the animals used in the chronic experiments. The myogram was recorded by implanting electrodes in the neck muscles. The reference electrode was fixed in the nasal bones. The animals were used in the experiments 7-10 days after the operation. Behavioral responses of the rats were recorded as motion pictures. The general motor activity of the animals was assessed quantitatively by the method of total actometry, consisting of counting oscillations of a seismic transducer, fixed in the moving floor, by means of an F5264 counter during time intervals of between 2 and 10 min, assigned by an F5235 transcriber, followed by presentation of the data on an "Optex" digital printer, and also by the open field method [1], in which the number of boundaries between squares crossed in 2 min of observation was counted as the experimental animal moved in the center of the field. Muscular rigidity was determined by the resistance felt during passive abduction of the limb. As the index of rigidity of the trunk muscles we used the degree of lordosis of the animal, estimated as the reciprocal of the distance from the head to the base of the tail during the period when the animal ceased to move [5]. The myogram was estimated quantitatively by counting discharges during a 5-sec interval by means of an F5264 counter, to the input of which electrical signals were transmitted from a UBP 2-03 amplifier.

Microinjection of DSIP (synthesized at the M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR) was given in a volume of 1-μl physiological saline and in a dose of 5-10 nmoles bilaterally into the unrestrained animals through previously implanted cannulas. Under the same conditions 1 μl of physiological saline alone was injected into animals

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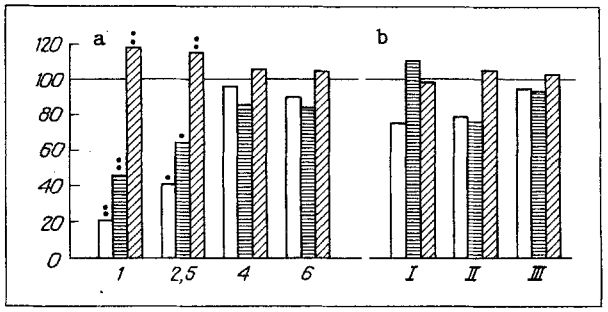

Fig. 1. Effect of injection of DSIP into SN on motor activity and muscle tone. a) Time course of effect of DSIP injection in a dose of 10 nmoles (abscissa, time, in h, after bilateral injection of DSIP; ordinate, parameters studied in percent of initial level, taken as 100%, and indicated by horizontal line); b: I) 1 h after injection of physiological saline (1 μl) into SN; II) 1 h after injection of DSIP (5 nmoles) into SN; III) 1 h after injection of DSIP (10 nmoles) into caudate nuclei. Unshaded columns — general motor activity, horizontally shaded columns — number of boundaries between squares crossed, obliquely shaded columns — muscle tone (reciprocal of distance from occiput to base of tail); one dot indicates $p < 0.05$, two dots indicate $p < 0.02$.

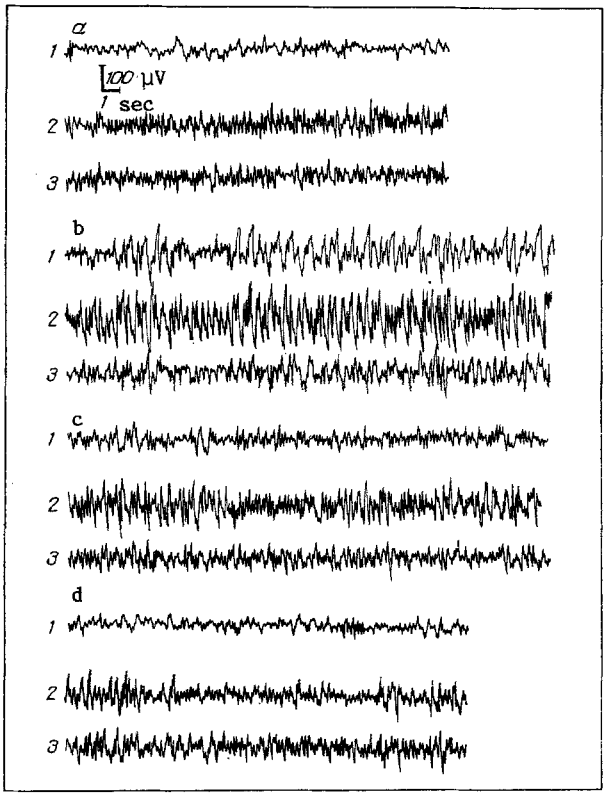

Fig. 2. Effect of injection of DSIP into SN on EEG of a rat. a) Initial EEG activity; b, c, d) 6, 45, and 85 min respectively after bilateral injection of DSIP (10 nmoles) into SN. 1) Sensomotor cortex, 2) caudate nucleus, 3) SN of left hemisphere. Calibration 100 μV, time marker 1 sec.

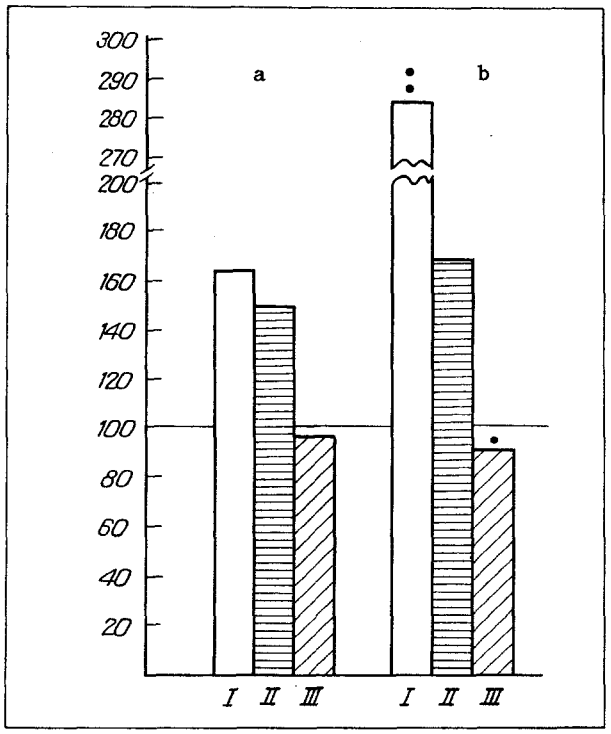

Fig. 3. Effect of benzhexol (a — 1 mg/kg, b — 5 mg/kg) on manifestations of parkinsonian syndrome caused by injection of DSIP into SN. Abscissa: I, II, III) parameters of general motor activity, number of boundaries between squares crossed, and muscle tone (in % of their initial level, taken as 100%, and indicated by a horizontal line); one dot indicates $p < 0.05$, two dots $p < 0.01$.

of the control group. Benzhexol was injected intraperitoneally in doses of 1-5 mg/kg. At the end of the experiments the location of the tips of the cannulas and electrodes was verified histologically. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Total cessation of movement of the rats for between 10-20 sec and 5-6.5 min was observed 3-10 min after injection of DSIP (10 nmoles) into SN. The animals developed the characteristic lordosis (the distance from the occiput to the base of the tail was significantly reduced compared with animals of the control group: 11.5 ± 0.3 and 13.3 ± 0.3 cm respectively; $p < 0.01$ — Fig. 1a). During passive abduction of the limbs, considerable resistance was felt. In the resting animals an increase in the number of discharges in the electromyogram by 30-40% was observed compared with the initial level of electromyographic activity ($p < 0.01$). When the rats moved into the center of the open field four of the 10 animals remained there throughout the period of observation (2 min). The number of boundaries between squares crossed by the animals fell significantly compared with the control group, and also compared with the initial value of this parameter in animals of the experimental group, which fell from 27.3 ± 2.5 to 10.9 ± 4.5 squares ($p < 0.02$; Fig. 1a) correspondingly. The parameter of general motor activity also fell significantly compared both with the initial background value (from 547 ± 130 to 133 ± 46; $p < 0.02$) and with the analogous parameter in animals of the control group (580 ± 110; Fig. 1a). In the period of commencing locomotion of the rats slowing and rigidity of the limb movements were observed with a decrease in the length of the step and the development of high tone of the tail, which curved like an arch and was held high above the spine. These behavioral changes were observed for 60-80 min after microinjection of DSIP, after which their severity gradually decreased and after 3.5-4 h no difference could be found between the parameters compared with the initial period and with the control (Fig. 1a). During this period the rats moved freely, sniffed the floor and walls of the chamber, and stood up on their hind limbs.

The aim of a separate series of experiments was to study behavioral reactions in rats to injection of DSIP into SN in a dose of 5 nmoles, and also to injection of DSIP into the caudate nuclei in a dose of 10 nmoles. When DSIP was injected under these conditions, no significant changes were found in the motor activity and muscle tone of the animals compared with the initial background and with the control (Fig. 1b).

In five of eight rats paroxysms of slow-wave activity with a frequency of 2-5 Hz and an amplitude of between 150 and 250 μV were recorded in the caudate nuclei on the EEG during the motor disturbances described above (2-10 min after injection of DSIP). Slow-wave activity also was recorded (but was weaker) in the cerebral cortex and it was absent in SN (Fig. 2b, 1 and 3). These paroxysms had a duration of 5-10 to 30-40 sec and alternated with short periods (5-20 sec) of thetalike activity. Slow-wave activity in the caudate nuclei diminished 30-60 min after injection of DSIP: the duration of the periods of generation of slow-wave discharges was 5-15 sec, but the frequency of their appearance was 1-5 times per minute (Fig. 2c, 2). After a further 30-40 min the slow-wave activity described above was absent in the caudate nuclei (Fig. 2d). In animals of the control group (bilateral injection of physiological saline into SN) no such changes in electrical activity were observed in the brain structures mentioned above. The study of the effects of benzhexol on manifestations of the parkinsonian syndrome showed that intraperitoneal injection of the drug in a dose of 5 mg/kg at the height of its development caused the motor activity of the animals to be increased from 202 ± 58 to 576 ± 93 oscillations of the transducer ($p < 0.01$) and a significant reduction of the increased muscle tone (an increase in the head – base of tail distance from 11.5 ± 0.3 to 12.7 ± 0.3 cm ($p < 0.05$; Fig. 3)).

The investigations thus showed that injection of DSIP into SN of rats leads to the formation of oligoakinetic changes, and also to the appearance of muscular rigidity, namely, the akineticorigid form of the parkinsonian syndrome. These changes appeared in the animals immediately after injection of DSIP and they were observed for 4 h, evidence of the acute character of the postural-tonic and motor disturbances induced by it. During the development of the parkinsonian syndrome in the animals, marked slow-wave activity developed in the zone of the caudate nuclei, indicating the formation of a GPEE in the caudate nuclei, determining the development of this neuropathological syndrome [3]. It will be noted that weakening of the manifestations of the syndrome in the animals also took place parallel to the decrease in intensity of slow-wave activity in the caudate nuclei. It can be tentatively suggested that activation of the caudate nuclei and the formation of a GPEE in them during injection of DSIP into SN takes place as a result of disinhibition of neurons in the caudate nuclei and is connected with abolition of inhibitory nigrostriatal influences. Reduction of dopamine release by nigrostriatal terminals may be due to strengthening of GABA-ergic inhibition, which has been observed during the use of DSIP [7], and also with activation of autoreceptors of dopaminergic neurons as a result of a fall in activity of type B monoamine oxidase [2]. Incidentally, the pathological determinant of the syndrome described above, like that of other forms of parkinsonian syndrome, consists of cholinergic neurons, as is shown by the efficacy of benzhexol treatment.

The action of DSIP, however, can also be effected through opiatergic mechanisms. It has been shown that agonists of kappa-opiate receptors cause the development of akinetic rigidity when injected into SN [8], and the rotation syndrome induced by injection of opiates into SN is not blocked by haloperidol [10].

These investigations suggest that an increase in the DSIP concentration in SN, inducing the formation of a pathological determinant in the caudate nuclei, may be one of the pathogenetic mechanisms of development of the parkinsonian syndrome.

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