EFFECT OF SUBSTANCE P ON AN EXPERIMENTAL PARKINSONIAN SYNDROME

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It is generally recognized that dopamine (DA) insufficiency in the caudate nuclei (CN) of the brain is a condition for the development of parkinsonism. DA deficiency in CN has been shown to disinhibit the neurons of CN and to form a generator of pathologically enhanced excitation (GPEE) from them, and that its duration determines the development of the principal manifestations of the parkinsonian syndrome [1, 3]. There is reason to suppose that besides DA deficiency, an imbalance of other neurotransmitters also plays a role in the pathogenesis of the parkinsonian syndrome. The presence of intimate functional connections between the nigrostriatal dopaminergic neurons and strionigral neurons containing substance P (SP) [6, 8, 11, 14], raises the question of whether changes in SP activity are involved in the pathogenesis of parkinsonism.

The aim of this investigation was to study the effects of SP, injected into CN, on the electrophysiological and behavioral manifestations of experimental parkinsonism, caused by systemic injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [3, 9, 10].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats aged 12 months and weighing 500-650 g. Under hexobarbital anesthesia, metal cannulas (for injection of SP) with nichrome electrodes (to record electrical activity – EA) fixed to them, were implanted bilaterally into the rostral zones of CN. The reference electrode was fixed in the nasal bones. Silver electrodes were implanted at the same time into the sensomotor cortex (SMC). The animals were kept in individual cages under standard animal house conditions on an ordinary diet. EA was recorded a week after the operation in unrestrained animals. MPTP hydrochloride, in a dose of 10 mg/kg, was injected twice a day for 4 days after the day of recording spontaneous EA, with intervals of 12 h (the total dose of the neurotoxin was 80 mg/kg). Oligokinesia, rigidity, and tremor were assessed in points [3]. Four days after the beginning of MPTP injections into the animals, in which marked signs of a parkinsonian syndrome was present, 5 μg of SP ("Serva, West Germany) was injected through the cannulas implanted into the rostral zones of CN bilaterally in a volume of 2 μl and at the rate of 1 μl/min. Animals of the control group, with similar symptoms of parkinsonism, received injections of the same volume of physiological saline. Motor disturbances and EA were recorded before and for 4 h after injection of SP. In another series of experiments SP was injected intraperitoneally in doses of 125 and 250 μg/kg. The corresponding volume of physiological saline was injected intraperitoneally into the animals of the control group in this series. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As was described previously [3], repeated intraperitoneal injections of MPTP into rats lead to the development of a parkinsonian syndrome, whose severity depends on the dose of the compound and the age of the animals. In most (26 of 34) animals, 4 days after the

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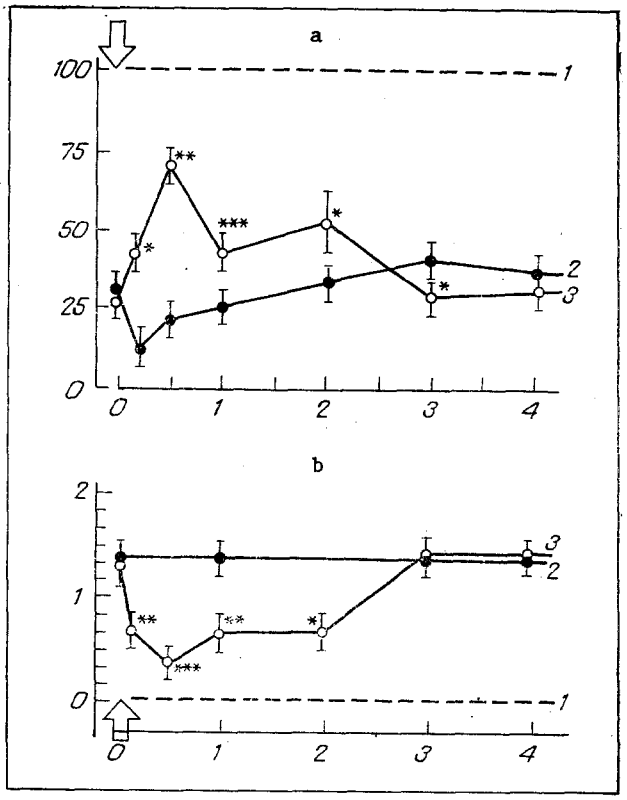

Fig. 1. Effect of intracaudate injection of substance P (SP) on oligokinesia and rigidity induced by systemic injection of MPTP. Abscissa, time of observation (in h) after injection of SP; ordinate: a) motor activity (in %), number of squares crossed and number of rearings in intact animals, taken as 100%, rigidity (in points). 1-3) Severity of phenomena: 1) in intact animals, 2) in control rats (injection of physiological saline into CN), 3) in experimental animals (injection of MPTP + SP into CN). Asterisks indicate significance of differences compared with control: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Arrow indicates injection of MPTP.

beginning of MPTP injections, a well-marked degree of oligokinesia was observed (up to 10-25% compared with intact rats), namely considerable slowing of movements, quantitative and qualitative diminution of motor activity of different kinds, a marked decrease in the number of squares crossed in the open field test, with disappearance of vertical movements (rearing), investigative activity, and grooming. Muscular rigidity was less marked (1.5 points) (Fig. 1a, b). Besides motor disturbances, most animals also showed a moderate decrease in body weight and hypothermia.

Bilateral injection of SP into the rostral zones of CN induced a marked increase of motor activity in all 16 experimental animals with oligokinesia and rigidity (Fig. 1a). This effect appeared immediately after injection of the neuropeptide and was strongest in the first 30 min, and decreasing during the next 30 min, but still remaining significantly higher than in the control. A second, spontaneous, but smaller increase in the animals' motor activity than in the first 30 min was recorded 2 h after injection of SP. Later a gradual decrease of motor activity was observed, and 3 h after injection of SP it could even be somewhat less than in the control animals, but after 4 h it was the same as in rats of the control group.

Parallel with the above-mentioned weakening of oligokinesia in the animals a marked reduction of rigidity also was observed, and was most evident during the first 30 min (Fig. 1b). Later the rigidity increased, but remained weaker than in the control rats for 2 h, and after 3 h it was virtually identical in the experimental and control animals.

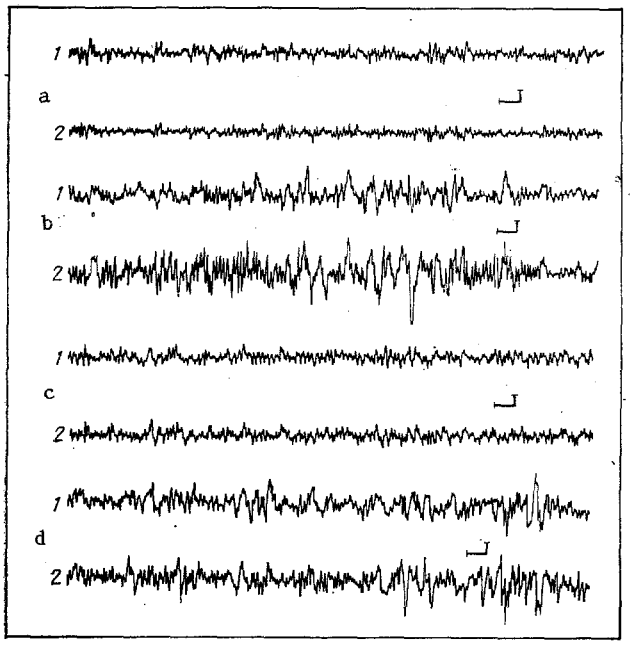

Fig. 2. EA recorded in sensomotor cortex (1) and CN (2), before (a) and on 5th day (b) after beginning of injection of MPTP (rat with marked oligokinesia and rigidity), and 30 min (c) and 3 h (d) after intracaudate injection of SP in the same rat. Calibration: 50 μV, 1 sec. Explanation in text.

Spontaneous EA (before injection of MPTP) in CN and SMC was characterized by dysrhythmia and the presence of low-amplitude (30-60 μV) fast and slow waves with periodic theta-like activity (Fig. 2a). Five days after the beginning of injection of MPTP, paroxysmal discharges of high-amplitude fast and slow waves were recorded in CN and SMC of animals with marked oligokinesia and rigidity. Grouped high-amplitude slow waves with a frequency of 0.5-1 Hz and an amplitude of more than 200 μV also were observed. The changes observed were more marked in CN, in which the amplitude of the slow waves could reach 600 μV, and the paroxysmal discharges and high-amplitude slow waves in CN were of longer duration (12-14 sec) than in the cortex (4-6 sec) (Fig. 2b).

Changes of EA in CN and SMC developed virtually immediately after microinjection of SP into the rostral zones of CN and were expressed as a reduction in the number and duration of the paroxysmal discharges (from 3-5 sec in CN and from 2 sec in SMC), and lowering of their amplitude; a decrease in the frequency and amplitude of the slow waves also was observed (to 100-150 μV in CN and to 150-200 μV in SMC). Almost complete normalization of EA occurred 30 min after intrastriatal injection of SP, both in CN and in SMC (Fig. 2c). Single and short (1-2 sec) paroxysmal discharges and slow waves, both of average amplitude, began to appear 1 h after injection of SP. After 3 h, the increased paroxysmal activity was restored, and it differed only a little from EA before injection of SP: At this time grouped high-amplitude (up to 300 μV) fast and slow waves with a frequency of 1-2 Hz and an amplitude of 600 μV reappeared, and were most marked in CN (Fig. 2d).

In the control experiments (six animals) intrastriatal injection of physiological saline had no significant effect on EA of either SMC or CN, or on the behavioral phenomena of parkinsonism.

Intraperitoneal injection of SP in doses of 125 and 250 μg/kg had no significant effect on the time course of EA in SMC and CN, or on the behavior of animals with a parkinsonian syndrome.

Comparison of changes in the clinical and electrographic manifestations of the parkinsonian syndrome after injection of SP into CN revealed correlation of these changes in time and in their dynamics: weakening of oligokinesia and rigidity, and disappearance of the enhanced paroxysmal EA in CN and SMC began immediately after injection of SP and were most marked during the first 30 min; the appearance of oligokinesia and rigidity corresponded to restoration of the paroxysmal EA in SMC and CN 3 h after injection of SP.

Thus the investigations showed that injection of SP into the rostral part of both CN, into the region of localization of the GPEE, induces suppression of activity of the latter and marked weakening of the phenomena of parkinsonism, namely oligokinesia and rigidity. The primary pathological determinant of all three basic symptoms of parkinsonism (oligokinesia, rigidity, and tremor) consists of the structures of the hyperactive CN in which the GPEE was formed; their suppression by intrastriatal injection of DA [4] or of other drugs [2] leads to disappearance of the pathological systems [1] of the above-mentioned symptoms and to their clinical disappearance. The same effect also was obtained in the present investigation. Suppression of GPEE in CN after intrastriatal injection of SP was unconnected with any mechanical effect, for injection of physiological saline into the region of the GPEE caused no significant inhibition of activity of the GPEE or disappearance of the oligokinesia and rigidity. The fact that SP, when injected intraperitoneally, does not give the suppressive effect mentioned above can be explained by metabolic degradation of SP and by the fact that it does not reach the nigrostriatal structures.

There is evidence that after injection of MPTP into animals, the content of SP in the substantia nigra (SN) increases [5, 12]. This effect has not been explained; it is perhaps an expression of compensation. According to clinical evidence, in human parkinsonism immunoreactivity to SP is increased in SN [7], whereas according to other data [13, 15] the SP concentration in SN is reduced in patients with parkinsonism. The problem as a whole requires further research. The results of the present investigation suggest that inadequate efficacy of SP in the nigrostriatal system may be a pathogenetic component of parkinsonism. Elucidation of the mechanisms of suppression of neuronal hyperactivity (GPEE) in CN after injection of SP into them is particularly interesting: it is due to the direct action of SP on hyperactive neurons or indirectly to inhibitory mechanisms (for example, through increased secretion of DA by striatal terminals of the nigral neurons, which has an inhibitory effect on the neurons of CN).

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