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CLINICAL AND ELECTROENCEPHALOGRAPHIC CHANGES IN MMP^+-INDUCED PARKINSONIAN SYNDROME IN RATS

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The parkinsonian syndrome induced by injection of tetanus toxin [1], cainic acid [3], acetylcholine [5], and antibodies to dopamine (DA) [6] into the caudate nuclei (CN), and also by systemic injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [4], which damages DA neurons of substantia nigra (SN), is connected with the formation for a generator of pathologically enhanced excitation (GPEE) in CN [2]. Recent data indicate that the toxic effect of MPTP on neurons of SN is due to the end product of its oxidation, namely 1-methyl-4-phenylpyridinium (MPP^+), which has high affinity for DA neurons [9-11]. Injection of MPP^+ directly into SN induces marked degeneration of the DA neurons of SN, reduces the concentration of striatal DA and motor disturbances characteristic of the parkinsonian syndrome, in rats and mice [7, 12, 13].

The aim of this investigation was to compare the severity of behavioral disturbances in a parkinsonian syndrome induced by intranigral injection of MPP^+, and the EEG changes in various brain structures and to elucidate the generator mechanisms of this syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats aged 9-10 months and weighing 440-550 g. Under hexobarbital anesthesia, and taking coordinates from a stereotaxic atlas [8], metal cannulas with bipolar electrodes for recording electrical activity (EA) were implanted bilaterally into the compact zone of SN. Bipolar nichrome electrodes were implanted simultaneously in the rostral zones of CN, the globus pallidus (GP), and the ventrolateral thalamic nucleus (VLT). The animals were kept in individual cages under standard animal house conditions and on the ordinary diet. By means of a Hamilton microsyringe, 7-8 days after the operation 10 μg of MMP^+ in a volume of 2 μliters of physiological saline was injected once through the cannula into each nucleus of SN (MPP^+ consisted of yellow crystals, m.p. 164-165°C, and was characterized by its IR and PMR spectra). The corresponding concentration of sodium iodide in 2 μliters of physiological saline was injected into animals of the control gorup, for the iodide of 1-methyl-4-phenylpyridinium was used in the experiment. EA was recorded on a "Neurograph-18" electroencephalograph (O.T.E. Biomedica, Italy), and it was subsequently processed on a BAS-161 computer from the same firm. EA was recorded before injection of the neurotoxin, continuously for 5 h, and for 1 h daily during the first 3 days. EA was recorded and microinjections of MPP^+ given while the animals were unrestrained. Oligokinesia, rigidity, and tremor were assessed on a point system [4]. The experimental results were subjected to statistical analysis.

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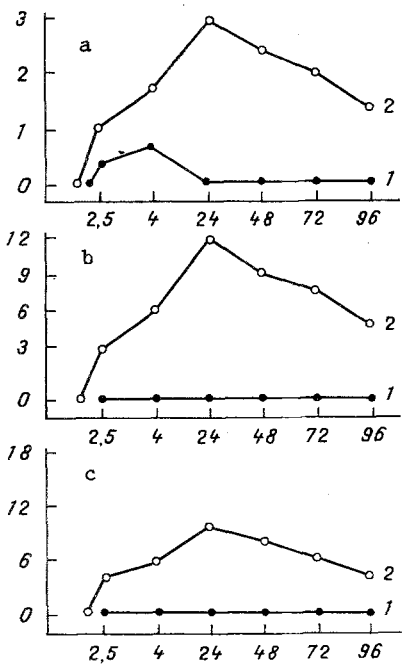

Fig. 1

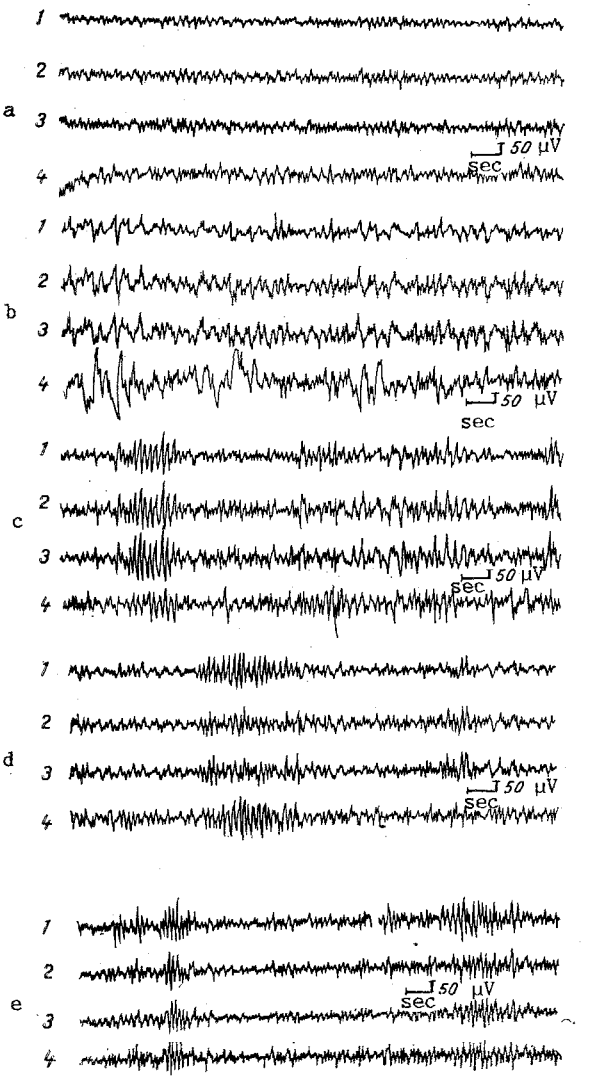

Fig. 2

Fig. 1. Time course of manifestations of parkinsonian syndrome in rats after intranigral injection of 10 μg MPP^+. Ordinate, severity of symptoms (in points); a) oligokinesia, b) rigidity, c) tremor; abscissa, time of observation (in h), $p < 0.05$. 1) Control, 2) experiment.

Fig. 2. EA of brain structures after bilateral injection of MPP^+ into SN. a) Control, b) 10 min, c) 40 min, d) 24 h, e) 72 h after injection of MMP^+. 1) CN, 2) GP, 3) VLT, 4) SN.

EXPERIMENTAL RESULTS

Bilateral injection of MPP^+ into the compact zone of SN induced a reduction of the animals' motor activity 5-10 min after injection of the neurotoxin. The first signs of rigidity appeared after 15-20 min in the form of increased muscle tone of the forelimbs, and rigidity of the trunk muscles appeared after 30-40 min in the form of slight kyphosis. A low-amplitude tremor of the head of short duration (5-15 sec), which lasted for 10-15 min, was observed in some rats 1 h after injection of MPP^+. During this period individual animals exhibited single shakings of the head of short duration. During the next 4-5 h muscular rigidity of the forelimbs and of the trunk muscles increased, and locomotor activity was considerably depressed.

The intensity of the motor disturbances of the animals was maximal during the first 48 h after injection of the neurotoxin. After 72 h the locomotor disturbances diminished, muscle tone was reduced, and rigidity (kyphosis) decreased. The time course of the parkinsonian syndrome after injection of MPP^+ into the rats is illustrated in Fig. 1. Besides extrapyramidal disturbances, all the experimental animals showed adipsia and aphagia 24 h after the injection, and they were well marked for the first 72 h after intranigral injection of

MPP^+. Reduction of the body weight of the animals, hypothermia, and changes in the fur cover also were observed.

EA of Test Structures. In the control animals dysrhythmic activity with fast, low-amplitude waves (12-14 waves/sec, 10-15 μV) and with irregular slow waves in the θ- and δ-bands, with an amplitude of 20-40 μV, was recorded in CN, GP, VLT, and SN (Fig. 2a).

Injection of MPP^+ (10 μg) into the compact zone of SN caused considerable changes in EA. During the first 5-10 min after injection of MPP^+ high-amplitude slow waves with split and pointed apices (2-3 waves/sec, amplitude up to 100 μV), and separate paroxysmal discharges of slow waves (1.5-2 waves/sec, amplitude up to 300 μV), with higher amplitude and longer duration in CN, appeared in all the structures tested (Fig. 2b). Paroxysmal activity (PA) became generalized 20-40 min after injection of MPP^+, the frequency of the waves of PA increased (5-6 waves/sec, amplitude 150-200 μV), and discharges of grouped waves appeared (Fig. 2c).

PA was reduced in GP and VLT 24 h after injection of MPP^+ but it was increased in CN (Fig. 2d). PA was reduced in GP, VLT, and SN 72 h after injection of the neurotoxin, but it still remained in CN. At this time diffuse slow activity in all the test structures also was reduced, but β-activity within the 14-15 waves/sec range was increased (Fig. 2e).

Computer analysis of PA showed that after intranigral injection of MPP^+, PA was most resistant in CN. During the first minutes after injection of the substance PA began to appear in SN (the beginning of the discharge occurred 60 msec before all other structures), but the greatest amplitude of PA was already noted at this time in CN: CN 256 μV, GP 168 μV, VLT 216 μV, and SN 184 μV. After 2-3 h PA began to be delayed in SN (the beginning of the paroxysm, indicated by the marker, was 300 msec in SN, 280 msec in VLT, and 200 msec in GP and CN) compared with other structures, i.e., at this time PA began earlier in CN than in SN and the thalamus, and it was higher in amplitude than in the other structures.

Analysis of these results shows that MPP^+ (the end metabolite of MPTP), if injected directly into the compact zone of SN, induces a whole symptom-complex (akinesia, rigidity, adipsia, and aphagia) characteristic of a lesion of this structure. Comparison of the electrophysiological changes with the clinical manifestations of motor disturbances shows that development of the principal symptoms of the parkinsonian syndrome (oligokinesia and rigidity) is connected with the formation of stable PA in CN. Weakening of the parkinsonian syndrome is accompanied by diminution of PA. Under these circumstances, PA is most stable in the electrical activity recorded from CN, and persists longer than in the other structures tested.

The data described above suggest that the development of parkinsonian manifestations following intranigral injection of MPP^+ is due to the formation of a generator of pathologically enhanced excitation in the striopallidal system. The fact that during decay of the process the GPEE persists longer in CN than in the other structures tested is evidence than CN is the determinant structure in the development of the parkinsonian syndrome.

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DETERMINATION OF LEADING FACTORS IN PATHOGENESIS OF ALCOHOL CARDIOMYOPATHY

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The aim of this investigation was to elucidate the leading mechanisms of damage to the rat heart during forced alcoholization.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 300-390 g, receiving dry food and water ad lib. Ethanol was injected into the stomach in the form of a 25% solution twice a day (at 9 a.m. and 9 p.m.) for 5.5 days. The doses of ethanol varied from 2 to 5 g/kg individually depending on the state of the animals and the time of routine injection [1]. When signs of intoxication were present, and allowing for their severity, the dose of ethanol was reduced. On the 4th day, 2 and 12 h after the 8th injection of ethanol, concentrations of ethanol, acetaldehyde, and acetone were determined [6] in blood taken from the caudal vessels. Mean 24-hourly concentrations of these compounds in the blood, the total dose of ethanol received by the rats during the experiment, and the index of tolerance of the rats to ethanol (ITE) were calculated, the latter by the formula $(A - 33)/(55 - 33)$, where A denotes the total dose of ethanol given in 5.5 days, (in g/kg), 33 is the conventional minimum total dose of ethanol (in g/kg); 55 the conventional maximal total dose of ethanol (in g/kg). The heart was removed from rats anesthetized with hexobarbital with the addition of heparin, 2-8 h after the last injection of ethanol, and perfused with Krebs–Henseleit solution containing 1% gelatinol, under a pressure of 70 mm Hg [5]. After 10 min of continuous perfusion the heart was switched to reperfusion with a volume of 35 ml of recycling solution, and this continued for 30 min. The peak systolic pressure (PSP) in the left ventricle, its components, the diastolic pressure (DP), the heart rate (HR) and the coronary flow (CF) were recorded. The pressures were measured by means of a "Statham" electromanometer, connected to a small latex balloon, inserted through an incision in the auricle of the left atrium into the left ventricle of the heart. The rate of contraction (RCH) and relaxation (RRH) of the heart and the tension time index (TTI) of the myocardium were calculated by the formula: $PSP \cdot HR \cdot T/1000$, where T denotes the tension time of the left ventricle. Creatine phosphokinase (CPK) activity was determined in the perfusion fluid [2]. At the end of perfusion the heart was dried to constant weight. Hearts of intact animals served as the controls. Student's test and Spearman's rank correlation method were used for the statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the method of alcoholization used in the investigation ensures a high, constant blood ethanol level and the appearance of acetaldehyde and acetone in the rat's blood. The mean 24-hourly acetaldehyde concentration correlated with the corresponding value of the blood ethanol concentration ($\rho = +0.572$, $p < 0.05$). ITE varied in different individuals from 0.32 to 0.82. Negative correlation was found between ITE and the residual blood ethanol concentration toward the time of the next routine injection ($\rho = -0.491$, $p < 0.05$). This agreement between individual doses of ethanol received by the rats on the 3rd day of the experiment and the mean-24-hourly levels ($\rho = +0.684$, $p < 0.01$)

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