EXPERIMENTAL PARKINSONIAN SYNDROME INDUCED IN RATS BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE

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An essential condition for the development of effective treatment of parkinsonism is the creation of a pathogenetically adequate model of this highly complex syndrome. It has recently been shown that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces various types of experimental parkinsonism in animals with all its three main components: akinesia, rigidity, and tremor [5]. These data explain the appearance of parkinsonism in patients who have taken for a long time the so-called synthetic heroin, which contains MPTP as an impurity [7]. The basic pathogenetic mechanism of the parkinsonian syndrome arising under the influence of MPTP is associated with degeneration of the dopaminergic neurons of the substantia nigra (SN), as a result of which a dopamine (DA) deficiency develops in the caudate nuclei (CN).

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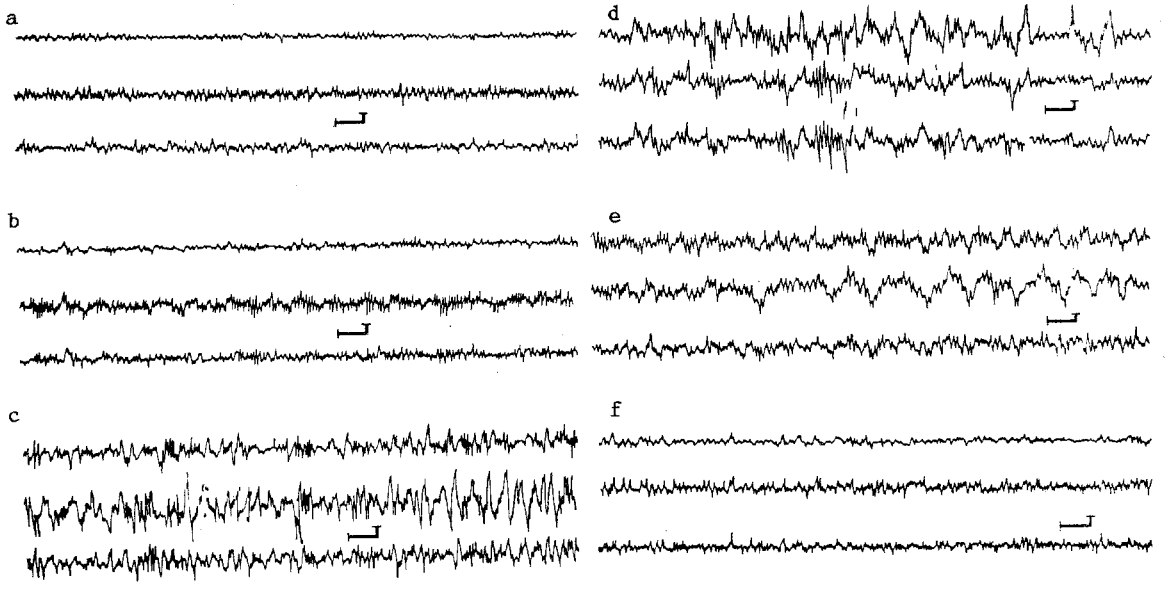

Fig. 1. EA recorded in sensomotor cortex (1), CN (2), and SN (3) in experiments with acute (a-c) and chronic (d-f) injection of MPTP during the development of a parkinsonian syndrome. Calibration: 50 μV, 1 sec. Explanation in text.

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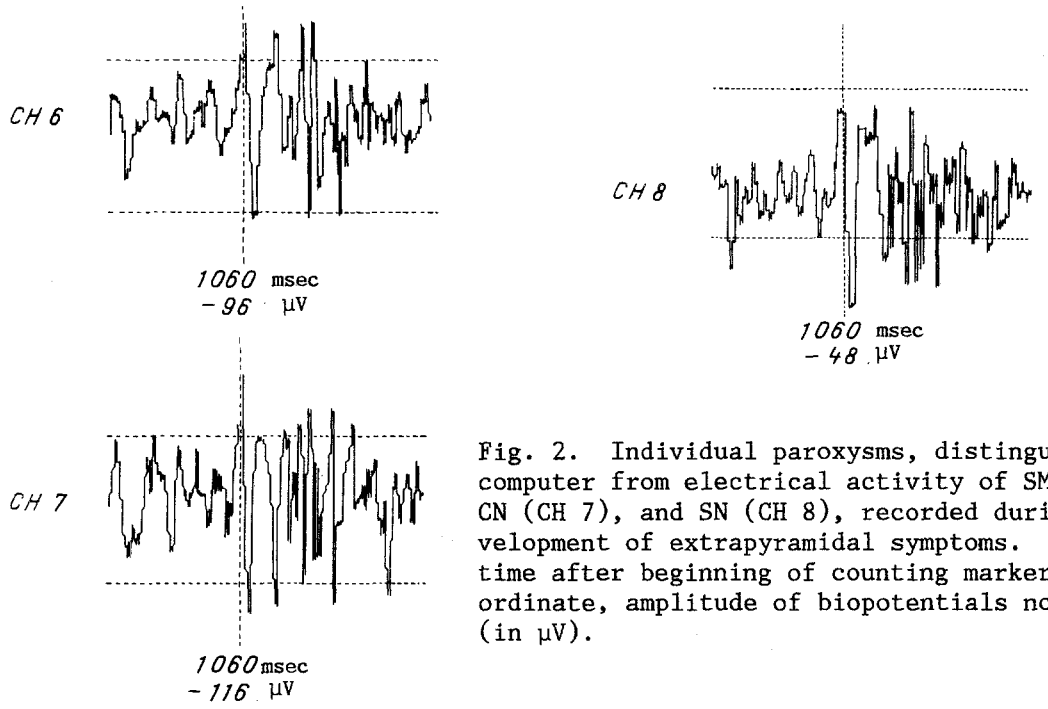

Fig. 2. Individual paroxysms, distinguished by computer from electrical activity of SMC (CH 6), CN (CH 7), and SN (CH 8), recorded during the development of extrapyramidal symptoms. Abscissa, time after beginning of counting marker (in msec); ordinate, amplitude of biopotentials noted by marker (in μV).

It was shown previously that a DA deficiency in CN caused by blockade of secretion by DA-terminals of nigral neurons under the influence of tetanus toxin leads to the onset of experimental parkinsonism [1], the immediate pathogenetic mechanism of which is the formation of a generator of pathologically enhanced excitation (GPEE) in CN [2, 3], because of dishinhibition of striatial neurons under conditions of DA deficiency. It might be supposed that in a parkinsonian syndrome caused by MPTP, a GPEE also would be formed in CN because of degeneration of the dopaminergic neurons of SN.

The aim of this investigation was to reproduce experimentally a parkinsonian syndrome with the aid of MPTP, synthesized by ourselves, and to study electrical activity (EA) in CN and also in the compact zone of SN and the sensomotor cortex (SMC) during the development of this syndrome.

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar albino rats weighing 220-350 g. MPTP hydrochloride (mp 253-254°C) was synthesized by methods in [4, 8] and characterized by elementary analysis, by IR-, PMR- and mass-spectra, and by thin-layer chromatography. The compound obtained was completely identical with the sample of MPTP obtained from Research Biochemicals Inc. (US). The compound was injected intraperitioneally in doses of 5 to 70 mg/kg in physiological saline. Animals of the control group received the corresponding volume of physiological saline. To record EA in SMC, CN, and SN monopolar nichrome electrodes (diameter of tip 70 μ) were implanted in accordance with coordinates taken from a stereotaxic atlas [9]. The reference electrode was fixed in the frontal bone. EA was recorded on a "neurograph-18" electroencephalograph (Biomedica, Italy), and subsequently processed on a BAS-161 neurocomputer made by the same firm. EA was recorded before injection of the compound and during the 3-5 h after injection. Later, EA was recorded daily for 1-2 weeks.

Motor activity (the degree of bradykinesia) of the animals was studied in the open field test, during which the number of squares entered by the rats, the number of rears on to its hind limbs, the number of inspections of holes, and the number of groomings were recorded for 5 min. To assess muscular rigidity (MR) the "lordosis" sign was used; its severity depended on the degree of MR and was determined as the shortening of the distance from the neck to the base of the animal's tail while immobile. Muscle tone of the hind limbs was determined as the degree of resistance to passive flexion and extension. Tremor was assessed visually and recorded by motion pictures, with determination of the frequency and amplitude of shaking of the head, forelimbs, and tail. All three signs, namely bradykinesia, rigidity, and tremor, were assessed as "plus signs." Autonomic disturbances also were recorded. The animals remained under observation for 3-4 weeks. The experimental results were subjected to statistical analysis.

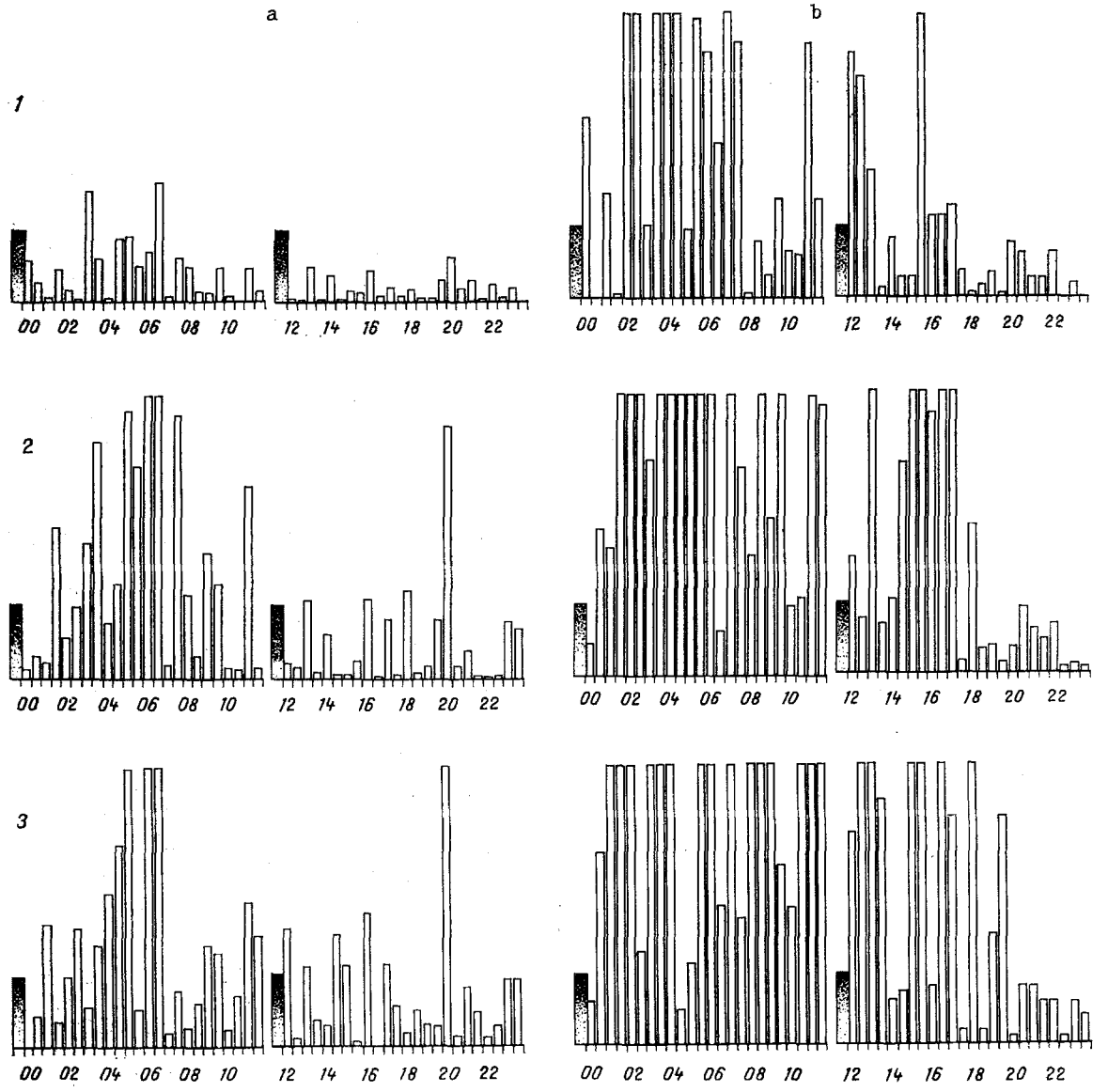

Fig. 3. Frequency power spectra of EA recorded in SMC (1), CN (2), and SN (3) in experiments with acute administration of MPTP. Abscissa, frequency of oscillations; ordinate, power of indvidual spectra (in μV^2). a) Background, b) during development of bradykinesia and rigidity.

EXPERIMENTAL RESULTS

Experiments carried out on 76 rats showed that LD_{50} of the compound for a single injection is 72.0 ± 3.1 mg/kg. A single injection of low doses (5-15 mg/kg) of MPTP caused a decrease of motor activity (+) in 13 of 18 animals for 20-60 min after injection. Brief low-amplitude tremor of the head (+) for 10-15 min was observed in individual animals. In response to a single injection of average doses (20-40 mg/kg) of MPTP, clonic convulsion appeared after 3-5 min in all 18 animals, and lasted 30-40 min. In the interictal period the animals' gait resembled that of an alligator. After the period of convulsions, a short period of motor hyperactivity was observed in some rats, with a rotation syndrome, retropulsion, and twitching of the head. All the animals developed autonomic disturbances (piloerection, disturbances of breathing, diarrhea, exophthalmos, salivation, etc.). Extrapyramidral disturbances appeared in 12 rats as a rule after the end of the period of convulsions, and were expressed as reduction and retardation of motor activity (++), episodes of "freezing," and transient low-amplitude tremor of the head (+) and rigidity (+). The bradykinesia of the animals persisted for up to 4 days.

A single injection of large doses (50-70 mg/kg) of MPTP caused clonic and tonic convulsions in all 18 animals, which lasted for 3-4 h. During this period six animals died. Twitching of the head and trunk, periodically accompanied by tremor of the head of average amplitude (++), marked bradykinesia (++, +++), and long episodes of "freezing," were observed in the remaining 12 rats after the end of the convulsions. Extrapyramidal disturbances were most marked during the 1st day after injection of MPTP and they persisted until 10 days.

In the experiments with chronic administration of average doses (30-40 mg/kg) of MPTP to 18 animals for 10 days, clonic convulsions and also the characteristic alligator gait of the interictal period were observed after each injection of the compound for 30-40 min. In response to repeated injections of the compound, the extrapyramidal bradykinesias became more marked. The animals' motor activity was reduced and retarded (++) and vertical movements, investigative responses, and grooming were absent. Twitching and tremor of the head (+), rigidity (+), autonomic disturbances, a decrease of body weight, and hypothermia were observed. These features persisted in the animals for 3-4 weeks after the end of administration of the compound.

In the rats of the control group, rhythmic activity was disturbed in all the structures tested, with the presence of slow waves (2-4 Hz, amplitude 30-70 μV) and of distinct fast EA (16-18 Hz, amplitude 10-15 μV). Regular EA with a frequency of 4-5 Hz appeared periodically (Fig. 1a).

The single injection of low doses of MPTP did not cause substantial changes in the EA. After single injections of average and high doses of the preparation a slowing down took place in the period of development of extrapyramidal symptoms; on the background of which appeared paroxysmal orders of high-amplitude fast and slow waves – the most expressed in CN and SMC (Fig. 2). With the appearance of bradykinesia, tremor, and rigidity, grouped waves were noticed on the electrogram (frequency 9-10 Hz; amplitude 50-70 μV) in all the studied structures (Fig. 1b). Intensification of the bradykinesia, tremor, and rigidity was accompanied by an increase in the slow activity. On the electrograms of all the studied structures at this time was registered high-amplitude slow activity of 1-2 Hz frequency and amplitude 200 μV (Fig. 1c; 2 and Fig. 3b, 2) which was most pronounced in CN.

In experiments with chronic administration of average doses of MPTP electrical activity recorded in animals with marked extrapyramidal symptoms (bradykinesia, periodic tremor of the head, rigidity, long episodes of "freezing") revealed paroxysmal discharges in all structures studied (Fig. 1d). Against the background of general disturbances of rhythm, groups of slow waves (Fig. 1e) with a frequency of 0.5-2 Hz and an amplitude of over 200 μV were observed in CN; these groups were longer in duration than in animals receiving a single injection of MPTP. Meanwhile these changes in EA were periodically replaced by high-amplitude irregular activity with predominance of fast waves (Fig. 1f).

The results of these investigations show that the appearance of a parkinsonian syndrome in rats depends on the dose of MPTP injected. Comparison of the clinical and electrographic data suggested that the parkinsonian syndrome was formed in these animals if injection of MPTP causes the appearance of paroxysmal discharges of high-amplitude pointed and slow waves on the EEG, most marked in CN. An increase in the severity of the extrapyramidal disturbances corresponds on the EEG to stable high-amplitude slow waves, also most marked in CN.

Analysis of EA of the brain structures studied thus indicates that the development of a parkinsonian syndrome is linked with the formation of a GPEE in CN. GPEE formation is connected with disinhibition of striatal cholinergic neurons as a result of DA deficiency in CN. This conclusion is in agreement with the results of investigations by other workers, who observed bradykinesia, rigidity, tremor, and other extrapyramidal disturbances, accompanied by a fall in the concentration of DA and its metabolities in the nigrostriatal system, during long-term systemic administration of MPTP to rats [6, 10]. Hyperactive CN, in which a primary GPEE was formed, constitute the pathological determinant of the syndrome. Under the influence of the pathogical determinant a pathological system extending to certain brain structures is formed, and this is reflected in the corresponding manifestations of parkinsonism.

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PREVENTION OF DISTURBANCES OF ELECTRICAL STABILITY OF THE HEART IN EXPERIMENTAL MYOCARDIAL INFARCTION BY ADAPTATION TO ANOXIA

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Recent investigations have shown that adaptation to periodic anoxia in a pressure chamber can prevent or limit disturbances of the electrical stability of the heart arising during stress and cardiac arrhythmia and fibrillation associated with acute ischemia, and can also abolish established disturbances of contractility and electrical stability of the heart due to postinfarction cardiosclerosis [4, 5]. However, the question of how adaptation to periodic anoxia affects the electrical stability of the heart in acute myocardial infarction (MI) has remained unstudied until recently. Accordingly, it was decided to compare the effect of preliminary adaptation to anoxia on disturbances of electrical stability and contractility of the heart usually observed in MI.

EXPERIMENTAL METHODS

Male Wistar rats weighing 320-350 g were divided into four groups: 1) control animals, 2) animals with experimental MI, 3) animals adapted to anoxia, 4) adopted animals with experimental MI. Adaptation to anoxia was carried out in pressure chamber at an "altitude" of 5000 m for 6 h daily for 5 days a week for 6 weeks. Experimental MI was produced by the method in [9] by ligaton of the descending branch of the left coronary artery. The animals were used in the experiments 2 days after coronary occlusion.

The experiments to evaluate the parameters of electrical stability of the heart was done on animals anesthetized with pentobarbital (50 mg/kg). In the first stage the reaction of the heart to stimulation of the peripheral end of the divided vagus nerve (pulse duration 2 msec, delay 5 msec, frequency 20 Hz) by means of an ÉSL-2 electronic stimulator, was investigated. After determination of the threshold strength of current inducing bradycardia, the responses to stimulation with a strength of 1, 2, 3, and 4 thresholds were determined consecutively. The ECG was recorded on a Mingograf-34 (Siemens-Elema, Sweden) and the effect

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