PARKINSON'S SYNDROME AFTER ACETYLCHOLINE INJECTION INTO THE CAUDATE NUCLEI

D. N. Kryzhanovskii, M. A. Atadzhanov,
T. A. Voronina, and L. N. Nerobkova

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The creation of a generator of pathologically enhanced excitation (GPEE) [2] in both caudate nuclei (CN) by microinjections of tetanus toxin or kainic acid into them [4] causes the development of Parkinson's syndrome. Parkinsonism arising after systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [3], which injures dopamine neurons of the substantia nigra, is also associated with GPEE formation in these nuclei. Injection of cholineric agents into CN [7, 8, 10] and systemic administration of the cholinesterase inhibitor galanthamine [5] also induces Parkinson's syndrome.

The aim of this investigation was to determine whether Parkinson's syndrome, caused by injection of acetylcholine (ACh) into CN, is due to the formation of a GPEE in them and to compare activity of the GPEE with the clinical manifestations of the syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 250-350 g. The animals were kept under standard animal house conditions on an ordinary diet. Under hexobarbital anesthesia, metal cannulas with nichrome recording electrodes to record electrical activity (EA) were inserted bilaterally into the rostral zones of CN, taking coordinates from a stereotaxic atlas [9] of the rat. Electrodes were inserted simultaneously into the sensomotor cortex. The reference electrode was fixed in the nasal bones. Six days after the operation, 1-10 μg of ACh in a volume of 1 μl together with 1 μg of neostigmine in a volume of 2 μl was injected by means of a "Hamilton" microsyringe into each nucleus. Animals of the control group received an injection of the same volume of physiological saline. Oligokinesia, rigidity, and tremor were assessed on a point system (for details, see [3]). Tremor was recorded by means of a tremorograph, based on piezoelectric transducers, and oscillations were recorded on a polygraph. EA was recorded on a "Neurograph-18" electroencephalograph (O.T.E. Biomedica, Italy), and subsequently processed on the BAS-161 neurocomputer manufactured by the same firm. EA was recorded before injection of the drugs and continuously for 3-5 h after injection. Dopamine (DA) in a dose of 200 μg and in a volume of 4 μl was injected (at the rate of 1 μl/min) into the rostral zones of CN. Benzhexol in a dose of 2-4 mg/kg was injected intraperitoneally. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Clinical Picture of the Syndrome. Microinjection of ACh with neostigmine caused a triad of basic motor disturbances characteristic of parkinsonism: oligokinesia, rigidity, and tremor. These phenomena were observed in all 42 animals and their severity depended on the dose of the drugs injected. A combination of 5 μg ACh and 1 μg neostimine was optimal. With these doses, 3-5 min after microinjection of the drugs, against the background of marked hypokinesia (++), a periodic, brief (4-5 sec), low-amplitude tremor of the head appeared with a frequency of 6-8 oscillations/sec (+), and rigidity of the muscles was slight (+). Later the

*Academician of the Academy of Medical Sciences of the USSR.

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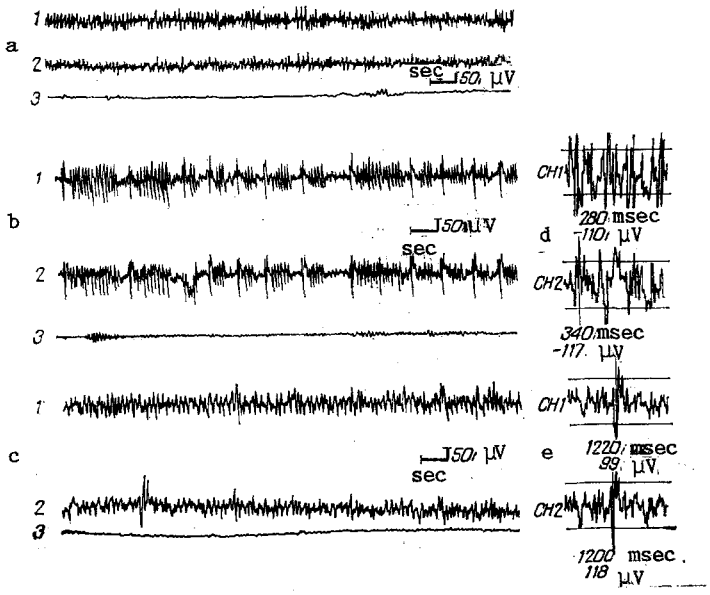

Fig. 1. EA of SC (1) and CN (2) and tremorogram (3) after bilateral injection of ACh with neostigimine into rostral zones of CN. a) Before injection of ACh with neostigmine, b) 10 min, d) 2.5 h after injection of these drugs. Computer analysis of discharges of SC (CH1) and CN (CH2) 10 min (c) and 2.5 h (e) after injection of ACh with neostigmine; msec) time after beginning of counting marker, μV) amplitude of potentials indicated by marker.

duration of the periods of onset of tremor was lengthened to 30-35 sec, and they were joined by low-amplitude tremor of the forelimbs (++). After 10-15 min tremor of the head and forelimbs of average amplitude together with low-amplitude tremor of the whole trunk appeared (+++). The duration of the periods of tremor lengthened to 3-5 min, whereas the pauses between them were shortened to 2-3 sec. Generalized tremor (+++) lasted 20-40 min, and low-amplitude tremor of the forelimbs and head (+) up to 1.5-2 h. On the disappearance of tremor, the clinical picture was dominated by akinetic-rigid manifestations. At this time the animals showed a sharp decrease of motor activity (+++) and marked rigidity of muscles of the hind limbs and trunk (++). The animals set with their spine curved into an arch, with the dipped head drawn toward the trunk. With the course of time the rigidity gradually weakened and it disappeared after 2-2.5 h. In the period of development of clinical symptoms, most animals also exhibited autonomic disturbances (hypersalivation, diarrhea, etc.).

Changes in EA. In intact rats, electrical activity of CN and the sensomotor cortex (SC) of intact rats revealed a disturbance of rhythmic activity with the presence of fast and slow waves, together with periodically appearing theta-activity (Figs. 1a, 2a, and 3a). After injection of ACh with neostigmine, characteristic changes took place in EA. Paroxysms of hypersynchronized activity were observed in CN and SC, in the form of grouped discharges 1-6 sec in duration. They consisted of complexes of high-amplitude pointed waves (spikes) and slow waves with an amplitude of 200-300 μV, grouped with discharges of synchronized waves with a frequency of 9-10 Hz, and with pointed peaks of lower amplitude (150-200 μV). During this time tremor was observed (Figs. 1b, 2b, and 3b). Pauses appeared between the grouped discharges in the form of low-amplitude activity; during these pauses the tremor decreased considerably.

In the stage of marked oligokinesia (+++) and less marked (+) rigidity (after 2-3 h) predominance of disturbed rhythmic activity with the presence of single slow (3-4 Hz) waves and with split apices (50-60 μV) and fast waves in the 12-14 Hz band; EA in the 9-10 Hz band was inhibited. These changes in EA corresponded to the almost total absence of tremor. Recordings of electrical activity showed the appearance of single high-amplitude slow waves with a frequency of 2-3 Hz and with an amplitude of 200-300 μV. They were recorded synchronously in both structure, but sometimes in CN only (Fig. 1d). Computer analysis showed that they appeared sooner in CN (Fig. 1e).

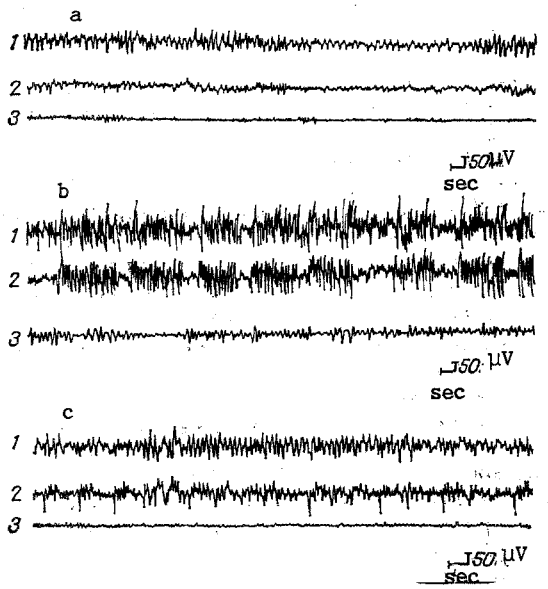

Fig. 2. Effects of DA when injected into CN. Electrical activity and tremorogram: a) before, b) 10 min after injection of ACh with neostigmine, c) 15 min after DA, injected 20 min after injection of ACh with neostigmine. Remainder of legend as to Fig. 1.

Intracaudate injection of ACh with neostigmine into both CN thus causes the appearance of marked paroxysmal activity in them, evidence of the formation of a GPEE in these structures.

Cholinergic neurons of CN are known to be inhibited by DA, which plays the role of inhibitory mediator for them [11]. Injection of DA (200 μg in 4 μl in the course of 4 min) into the rostral zones of CN, into which ACh with neostigmine had previously been injected and EA recorded, caused an increase in the animals' motor activity after 5-10 min, significant reduction or even disappearance of rigidity, and marked reduction of tremor. These effects occurred simultaneously with significant changes in electrical activity of CN and SC: the hypersynchronized grouped discharges and also discharges of high-amplitude slow waves disappeared (Fig. 2c), but the diffuse slow activity remained.

Systemic (intraperitoneal) injection of the cholinolytic benzhexol (2-4 mg/kg) at the stage of a manifest Parkinson's syndrome caused disappearance of the tremor, a marked decrease of rigidity, and an increase in the animals' motor activity, but the hypokinesis did not disappear completely. Clinical weakening of the syndrome took place simultaneously with disappearance of paroxysmal activity and with marked inhibition of slow waves (Fig. 3c).

Comparison of the effects of benzhexol and DA showed that both substances induce appreciable normalization of the clinical and electrographic parameters. Meanwhile, systemic injection of benzhexol abolished both the paroxymal activity and the diffuse slow-wave activity, whereas after injection of DA mainly inhibition of paroxysmal activity was observed, and slow-wave activity was preserved.

Thus after intracaudate injection of ACh with neostigmine, a GPEE of hyperactive cholinergic neurons is formed in CN. The appearance of clinical features of Parkinson's syndrome is linked with its onset and activity. These data are in agreement with the results of previous investigations which show that Parkinson's syndrome is caused by the formation of a GPEE in both CN [1, 3, 4]. Reproduction of an experimental Parkinson's syndrome by injection of cholinomimetics into CN was achieved previously by other workers [7, 8, 10]. The present investigation showed that the pathogenetic mechanism of this form of the syndrome is the formation of a GPEE in CN.

With this model of the syndrome tremor appeared initially, and this can be interpreted as the equivalent of the tremorous form of Parkinson's syndrome, and signs of hypokinesia and

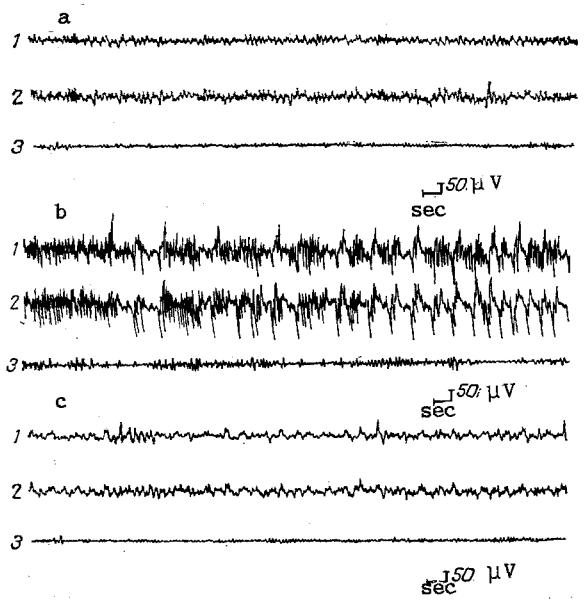

Fig. 3. Effects of systemic injection of benzhexol. Electrical activity and tremorogram: a) before, b) 10 min after injection of ACh with neostigmine, c) 20 min after intraperitoneal injection of benzhexol (2-4 mg/kg), injected 15 min after ACh with neostigmine. Remainder of legend as to Fig. 1.

rigidity, representing the akinetic-rigid form, appeared later. During tremor, synchronized activity with a frequency of 9-10 Hz predominated, and when that disappeared so also did the tremor. In the akinetic-rigid form of the syndrome EA was observed in CN mainly in the form of diffuse slow and fast waves. Further elucidation of relations between the character of EA in CN and the clinical features is of fundamental importance for the study of the pathogenetic mechanisms of the basic motor disorders in Parkinson's syndrome.

An examination of the pathogenesis of the complex Parkinson's syndrome from the standpoint of the generator, determinant, and systemic mechanisms of neuropathological syndromes [2] suggests that, as follows from previous experiments [1, 3, 4], the primary determinants of Parkinson's syndrome are hyperactive structures of CN, in which the GPEE is formed. This determinant group of structures induces the onset of pathological systems lying at the basis of the leading motor disorders in parkinsonism, namely tremor, hypokinesisa, and rigidity, directly or through the formation of secondary determinants. Each of these basic symptoms of parkinsonism has its own pathological system.

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