Involvement of the Striatal Serotoninergic System in Parkinson's Syndrome

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The dopaminergic nigrostriatal system, whose deficiency is of key importance in the pathogenesis of parkinsonism, interacts intimately with the serotoninergic system [10,13,15,19], and the reciprocal interactions between these two neurotransmitter systems at the level of the striatum - a brain structure pathogenetically significant for parkinsonism - dictate the approach to treatment of Parkinson's syndrome with agents that act on the mechanisms of serotonin transmission. The empirical clinical application of agonists and precursors of serotonin [9] or its antagonists [8, 14] has produced equivocal results. In order to elucidate the role which the striatal serotoninergic system plays in this syndrome, it is important to determine the effects of locally administered agents on striatal serotoninergic structures.

Since one mechanism in the pathogenesis of Parkinson's syndrome has been shown to be the activity of a generator of pathologically enhanced excitation (GPEE) arising in the caudate nucleus (CN) as a result of disrupted dopamine control over acetylcholine neurons [4], the present study was undertaken to see how the GPEE's activity and the manifestations of experimentally induced parkinsonism are affected by serotonin injected into the CN and by local inactivation of this serotonin with antibodies to it (SAb).

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MATERIALS AND METHODS

The experiments were carried out on an animal model of Parkinson's syndrome induced by intranigral injection of the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+), which damages the dopamine-synthesizing nigrostriatal neurons. A total of 46 Chinchilla rabbits weighing 2.5-3 kg and 28 randomly bred rats weighing 350-400 g were used. In the rabbits, MPP^+ was injected into the substantia nigra (SN) in a dose of 85 mg and a volume of 3 μl according to coordinates AP+5,LD3 of a stereotaxic atlas of rabbit brain [6]; serotonin (Serva) (50 μg/5 μl) and SAb (200 μg protein/5 μl) were injected into the CN according to stereotaxic coordinates AP-2,LD 4.5. Use was made of gamma globulins from rabbits immunized with the antigen serotonin-bovine serum albumin (S-BSA) obtained by a modification of the previously described procedure [18]. In control tests, gamma globulins obtained from BSA-immunized rabbits, containing equal amounts of protein and purified from anti-BSA antibodies, were used. The procedures for the immunization and the sedimentation and purification of gamma globulins were described in our previous study [5]. The substances used in this study were all dissolved in physiological saline immediately before use and were injected from a Hamilton microsyringe at a rate of 1 μl/min. Monopolar Nichrome electrodes were each implanted into the right and left CN of both test and control rabbits according to AP 0,LD6 coordinates; the indifferent

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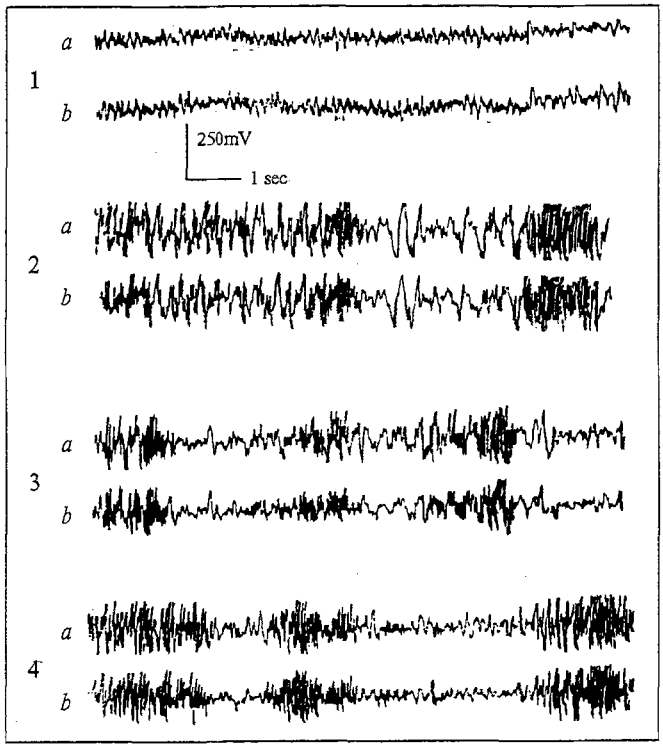

Fig. 1. Electrical activity of rabbit caudate nuclei (CN) after intranigral MPP^+ injection. *a*) right CN; *b*) left CN. *1*) baseline activity; *2*, *3*, and *4*) 1 h, 4 h, and 24 h, respectively, after MPP^+ injection.

electrode was fixed to a nasal bone. The edges of the operative wound were covered with a composite polymeric material (designed by Prof. M. V. Vogralik) to reduce the impact of the traumatic injury and achieve more reliable fixation of the electrodes. The stereotaxic operations were performed under Novocain anesthesia.

Tests were started when electrical activity in the CN returned to normal (7-10 days after the operation). The EEG was recorded on a Medicor encephalograph (Hungary). One hour after the injection of MPP^+ into the SN, serotonin and SAb were injected into the CN of rabbits in test groups № 1 and № 2, respectively; rabbits in test group № 3 were injected with gamma globulins without SAb, while those in control group № 4 did not receive any additional injections. Rabbits of control group № 5 were injected with physiological saline alone (control for mechanical trauma). Finally, test group № 6 received an intrastriatal injection of SAb.

Rats were injected, using coordinates of a stereotaxic atlas [17], with 50-100 μg of serotonin into the CN under ether anesthesia via an opening previously made in the parietal bone under Hexenal (hexobarbital sodium) anesthesia. Prior to serotonin injection and also 1 h and 24 h after it, "open-field" tests were carried out to measure the summated index of behavioral activity (SIBA), which takes into account the horizontal (number of squares crossed) and vertical (number of upright postures) movements, the number of times the animal looked into the "burrows", and the number of grooming acts. This index was expressed in percent relative to its initial value taken as 100%. Rats injected with a subthreshold intracaudal serotonin dose (10-30 μg) served as controls.

RESULTS

The normal focal bioelectrical activity in the rabbit CN is characterized by rapid low-amplitude (up to 20-40 μV) oscillations and irregular medium-amplitude (up to 60-110 μV) waves of the theta type (Fig. 1). Five to 15 min after the intranigral injection of MPP^+, groups of high-amplitude (up to 200-300 μV) oscillations in the delta and theta ranges appeared on the EEG. Later, at minutes 20-30, the EEG showed predominantly short (0.8-3 sec) paroxysmal discharges occurring at intervals of 2-4 sec and followed by much longer (85-300 sec) periods of

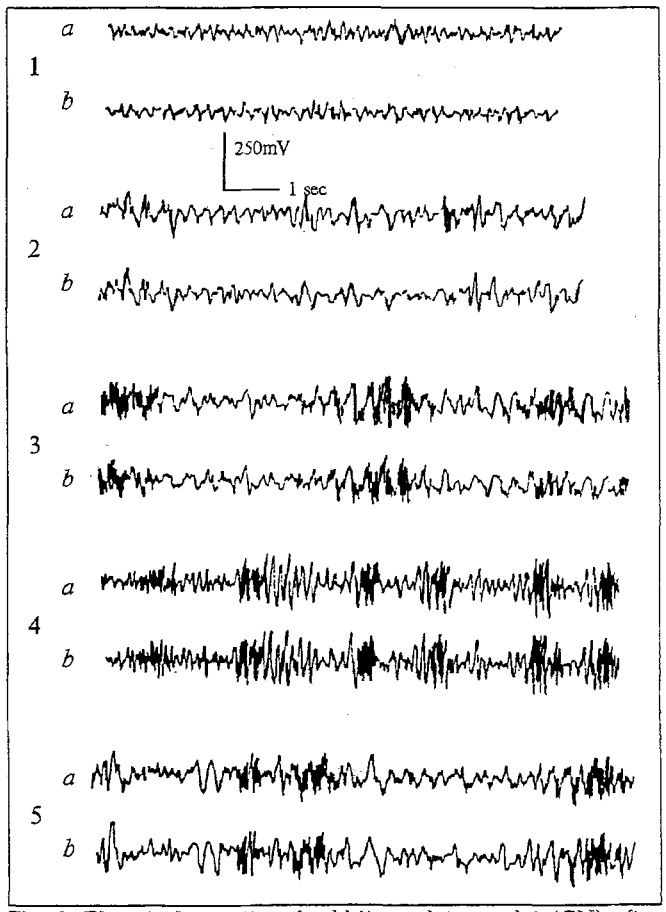

Fig. 2. Electrical activity of rabbit caudate nuclei (CN) after intranigral MPP^+ injection and intracaudate serotonin injection. *a*) right CN; *b*) left CN. *1*) baseline activity; *2*) 1 h after intranigral MPP^+ injection; *3*, *4*, and *5*) 5 min, 30 min, and 120 min, respectively, after intracaudate serotonin injection.

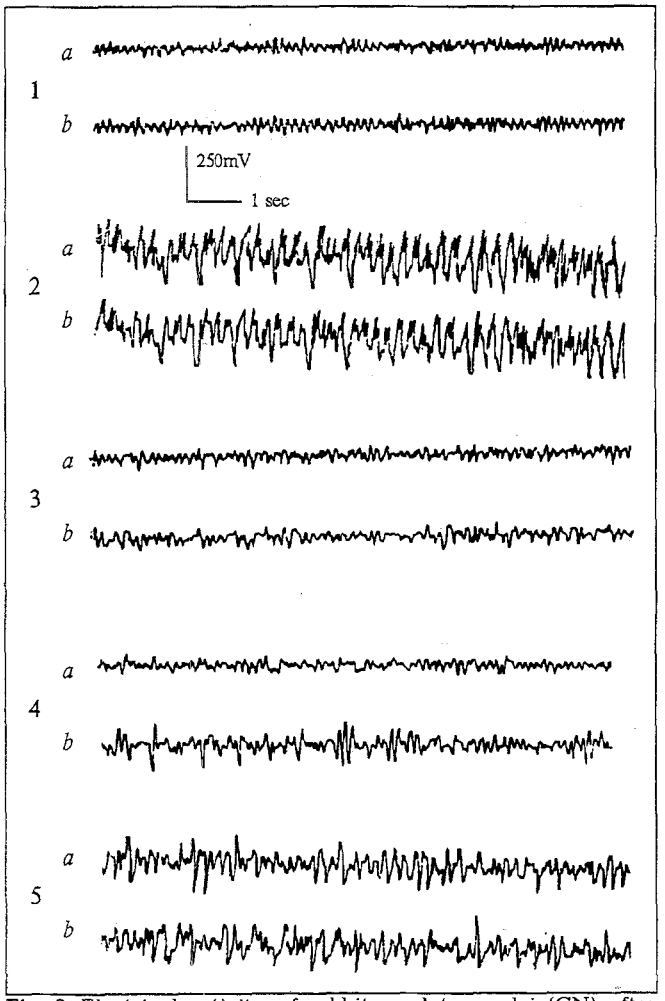

Fig. 3. Electrical activity of rabbit caudate nuclei (CN) after intranigral MPP^+ injection and intracaudate injection of serotonin antibody (SAb). *a*) right CN; *b*) left CN. *1*) baseline activity; *2*) 60 min after intranigral MPP^+ injection; *3*, *4*, and *5*) 5 min, 30 min, and 240 min, respectively, after intracaudate SAb injection.

high-amplitude activity (250-600 μV). In the short paroxysmal discharges, oscillations in the beta and theta ranges (12-18/sec and 4-6/sec, respectively) were recorded. During the above-mentioned prolonged periods, slow activities of the delta and theta types predominated. A high-amplitude activity during these periods was recorded continuously for 6 h. Short paroxysms were detectable for 24 to 48 h.

In the presence of the discharges described above, signs of parkinsonism were observed. The injection of MPP^+ led, after 40-60 min, to a greatly reduced motor activity. The rabbits remained immobile for 2-6 h and did not respond to tactile or strong acoustic stimuli. They retained their usual posture or assumed a posture associated with muscular rigidity (kyphotic posture). Muscular rigidity of the trunk, neck, and limbs was observed in 80% of the rabbits, while 45% of the animals developed a paroxysmal medium-amplitude tremor of the head and forelimbs.

In rabbits given physiological saline alone, an "injection effect" was observed, manifested in the appearance on the EEG of groups and short (6-20 sec) periods of medium- to high-amplitude (up to 150 μV) oscillations, followed by restoration of the initial activity.

The experiments described above were the first in which the MPP^+-induced parkinsonism syndrome was produced in a rabbit model. It should be noted, however, that MPP^+ is highly toxic to this species: some of the animals developed hypersalivation, acute rhinitis, and convulsions followed by rapid death. The data obtained for such rabbits were excluded from the analysis.

Shortly after the intracaudate serotonin injection, a greatly increased electrical activity was recorded in the CN (Fig. 2) and signs of Parkinson's syndrome were observed. During the first few minutes postinjection, all rabbits became akinetic and exhibited a strongly increased tonus of the masticatory musculature; 80% of them developed muscular rigidity of the trunk, neck, and limbs, while 45% exhibited tremor of the head. These symptoms of parkinsonism appeared earlier and were more severe than in rabbits injected with MPP^+ alone.

In rats, the intracaudate serotonin injection resulted in parkinsonism-like symptoms of short duration. All rats developed hypokinesia that persisted for 2.5-3 h; during the first 30-40 min postinjection, they remained immobile while retaining their usual posture. At 1 h postinjection, the SIBA in the open field was decreased to 65% of its initial level ($p<0.001$). During the first 40-60 min, rigidity of the trunk muscles (kyphosis) was observed, with a shortening to 1.5-3 cm of the distance between the neck and the root of the tail. In this period, a paroxysmal rigidity of the tail flexors characteristic of Jacob's serotonin syndrome was conspicuous, with individual paroxysms lasting from several seconds to 10-15 min; 45% of the rats also showed a paroxysmal tremor of the head at rest lasting for an hour or so. These observations agree with the reported data on the hyperactivation of CN structures [7,15] and the development of parkinsonism-like symptoms in intact animals following an intracerebral serotonin injection [11,15].

The injection of SAb at the stage when a GPEE was being formed in rabbits with strongly marked hypokinesia or rigidity led, in 100% and 50% of the animals, respectively, to weakened hyperactivity or its complete disappearance (Fig. 3), accompanied by decreasing rigidity or hypokinesia. The effect of SAb became manifest 5 to 30 min (16±3.4 min) postinjection and was in evidence for 1.2-3.5 h (2.3±0.3 h). Thereafter, groups of high- and medium-amplitude

oscillations appeared on the EEG, but no resumption of either hyperactivity or parkinsonism symptoms was observed in 62% of the rabbits; the remaining rabbits exhibited only one parkinsonism symptom - hypokinesia. In the control group injected with MPP^+ alone, the akineticrigid form of parkinsonism was observed in 62% of the rabbits, while the remaining animals developed all three major symptoms of the disease. Sometimes a fine tremor of the head was seen during the first few minutes after SAb administration, which appears to have been a response to the intracerebral injection of the protein, since a similar effect was noted in animals injected with a gamma globulin that did not contain antibody to serotonin.

The present results indicate that both electroencephalographic and clinical manifestations of Parkinson's syndrome are augmented by serotonin acting on CN structures and can be eliminated or reduced through selective inactivation of serotonin in this brain structure which plays an important part in the pathogenesis of parkinsonism. It may be speculated that the enhanced electrical activity which is displayed by the CN of rabbits injected with MPP^+ into this nucleus and which reflects increased GPEE activity occurs as a result of serotonin acting on CN neurons. Serotonin has been shown to be capable of depolarizing membranes [1, 19] and of potentiating stimulus-triggered intracellular processes [3]. Moreover, serotonin can reduce the release of dopamine in the striatum [20]. Intraventricular injection of serotonin antibody has been reported to diminish reserpine-induced akinesia and rigidity [16].

The pathogenesis of Parkinson's syndrome is likely to involve endogenous serotonin, which exerts stronger effects when dopamine is deficient in the CN. This possibility is strengthened by the reciprocal nature of the relationships between the serotoninergic mesostriatal system and the dopaminergic nigrostriatal system [2,13,15,19]. It is possible that in advanced age, when dopamine deficiency arises naturally [12], the impact of serotonin increases and the serotonin mechanisms begin to play a greater role in the pathogenesis of parkinsonism.

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