ANTIDOPAMINE ANTIBODIES IN THE PATHOGENESIS OF PARKINSONISM

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A factor of key importance in the pathogenesis of Parkinson's syndrome is insufficiency of the dopaminergic nigrostriatal system (DNSS) [10]. A previous investigation showed that an important pathogenetic mechanism of Parkinson's syndrome is the formation of a generator of pathologically enhanced excitation (GPEE) in the caudate nuclei (CN) [4] as a result of disinhibition of CN neurons due to dopamine (DA) insufficiency. It has been shown that GPEE and the triad of basic symptoms of parkinsonism, namely akinesia, rigidity, and tremor, can be reproduced by injection of DA-binding antibodies (AB) in the rat CN [5]. These data suggested that one factor in the development of insufficiency of the DNSS may be binding of DA by AB in the nigrostriatal system. The aim of the present investigation was a clinical and experimental study of the possible role of AB to DA in the pathogenesis of parkinsonism. The presence of AB to DA and to other neurotransmitters was determined in patients with parkinsonism, and the possibility of induction of Parkinson's syndrome by means of patients' gamma-globulins containing antidopamine AB was studied experimentally.

EXPERIMENTAL METHOD

Blood sera from patients with parkinsonism (91 persons) and from clinically healthy subjects (121) of the corresponding age, and who had undergone investigations in hospital, were tested for the presence of AB to L-dopa and DA by the passive hemagglutination test (PHT). A conjugate of DA and bovine serum albumin was used as the test antigen. The specificity of AB to DA and L-dopa was determined in the passive hemagglutination inhibition test (PHIT). The concentration of DA and L-dopa to inhibit the reaction was 10^{-4} mole/ml. The AB level (A) was expressed in conventional units and calculated by the equation: $A = T_1^{-1} - T_2^{-1}$, where T_1 denotes the titer of AB in PHT and T_2 their titer in PHIT. Depending on the presence or absence of DA-binding AB the patients sera were grouped and the gamma-globulin fraction (GG) isolated from them. Methods of synthesis of the test antigens and of isolation and purification of GG were described previously [5].

Experiments were carried out on noninbred male rats weighing 450-600 g, aged 10-12 months, and rats weighing 250-300 g, aged 3-5 months; the total number of rats used was 22. Under hexobarbital anesthesia, monopolar electrodes were inserted into the right and left CN under hexobarbital anesthesia, taking stereotaxic coordinates from an atlas of the rat brain [13]: AP = 0, L = 3, H = 4.5. The reference electrode was fixed in the nasal bone. Operations were carried out with the SEZh-5 stereotaxic apparatus. The main experiment was begun only after disappearance of injury discharges caused by insertion of the electrodes. GG were injected into the rats under ether anesthesia from a Hamilton microsyringe,

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TABLE 1. Detection of AB Binding L-dopa and DA in Blood Sera of Patients with Parkinsonism and Clinically Healthy Individuals

group	Number of subjects tested	Prevalence of antibodies, %	
		to L-dopa	to DA
Healthy young adults aged (20-30 years)	28	—	3,6
Healthy middle aged subjects (45-59 years)	27	25,9	48,1
Middle aged patients with parkinsonism			
untreated	24	25,0	54,2
treated with L-dopa preparations	17	52,9*	76,5*
Healthy elderly individuals (60-79 years)	31	25,8	51,6
Elderly patients with parkinsonism:			
untreated	35	25,7	40,0
Not determined	15	33,3	66,7*

Legend. Significant differences between untreated patients and those treated with L-dopa preparations, significant within the groups, are indicated by an asterisk ($p \leq 0.03$).

in a volume of 5 μl (200 μg protein) into CN, at a point 1 mm anterior to the site of the electrode tip. The effects of GG, isolated from patients' sera, containing and not containing AB to DA, were studied. Electrical activity in CN was recorded on a Uedicor FEG-8 electroencephalograph (Hungary) before injection of GG, immediately after its injection for a period of 5-6 h, and 24 h after injection. The behavior of the rats in an "open field (OF) was tested 3 times: before injection of GG and 1 and 24 h after injection. An integral parameter of the subsequent behavioral acts, performed during 4 min of testing, was analyzed: the number of squares crossed (horizontal movement), the number of rearings, the number of inspections of burrows, and the number of acts of grooming. The integral parameter of activity (IPA) was expressed as a percentage of the initial level. The significance of changes in IPA was determined by Fisher's test.

EXPERIMENTAL RESULTS

The results of tests on the human blood sera show that AB to DA as a rule are not found in healthy young adults (age 20-28 years), but are found equally often in elderly people, whether healthy or suffering from parkinsonism (Table 1).

Injection of GG isolated from blood sera of patients with parkinsonism and containing AB to DA into CN of rats aged 12 months led to the formation of a GPEE in CN. High-amplitude (250-300 μV) discharges of burst type, with predominance of waves in the theta-band, evidence of the presence of a GPEE, were recorded on the EEG of CN (Fig. 1). Besides the GPEE, the characteristic Parkinson's triad also appeared: oligokinesia (in 100% of cases), tremor (in 70%), and rigidity (in 50%). Tremor of low and medium amplitude appeared in paroxysms and was exhibited for 2-4 h; rigidity also appeared in paroxysms during the first 4-6 h after injection GG. The duration of the paroxysms varied from 2-5 sec to 2-3 min, and their appearance correlated with intensification of activity of the GPEE in CN. Oligokinesia was observed for 24 h: during the first 2-3 h after injection of GG the animals were almost completely immobile, and maintained their usual posture. IPA in of was 7.8% after 1 h and 17.5% after 24 h compared with the initial level. In the group of control rats receiving patients' GG not containing AB to DA the value of this parameter was 56.5% after 1 h and 58.5% after 24 h.

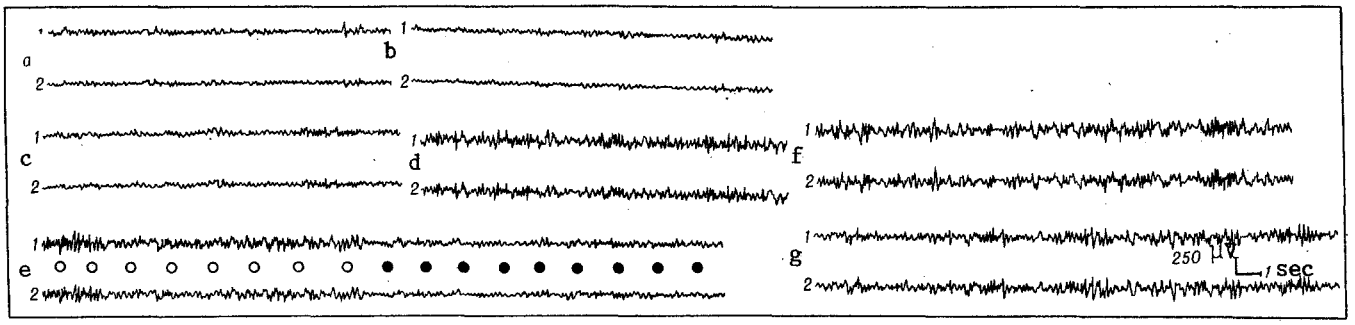

Fig. 1. Electrical activity in CN of rats after injection of GG, containing AB to DA, from patients with parkinsonism into CN: 1) right CN; 2) left CN; a) before, b-g) after injection of GG: b) 5 min, c) 20 min, d) 35 min, e) 50 min after injection: left fragment (empty circles) – animal immobile; right fragment (filled circles) – animal moves around the chamber; f) 1 h 20 min, g) 3 h 15 min after injection.

The pathogenic effect of GG containing AB to DA was manifested particularly clearly in rats of the older age group (10-12 months). Intrastriatal injection of the same dose of GG into young (3-5 months) animals caused significantly more intensive symptoms: oligokinesia occurred in 100% of cases, tremor in 50%, and rigidity in 25%.

Intracaudatal injection of GG, not containing AB and DA rom patients with parkinsonism into rats led to the development of oligokinosla (in 75% of cases), tremor (in 25%), and rigidity (in 25%). These symptoms were weaker than the effect of GG containing AB to DA: the value of IPA in OF was reduced by 45% 1 h after injection of GG and by 42% 24 h after injection, whereas in rats receiving an injection of GG containing AB to DA, IPA fell by 92 and 82% respectively ($p < 0.05$). Changes in electrical activity in CN during the investigation were not significant (Fig. 2).

The results of this investigation confirm the view that binding of DA by AB in the nigrostriatal system and, in particular, in CN, may be one condition for the onset of parkinsonism. Rats develop relative insufficiency of DNSS with age. Spontaneous increased electrical activity is recorded in CN of old rats, and paroxysmal hyperactivity may even be observed in some animals [1]; a Parkinson's syndrome is easily reproduced in old animals by injection of reserpine [9] and the neurotoxin MPTP [1]. To create a relatively active GPEE in CN in young animals, and thereby induce manifestations of parkinsonism [4], AB must be injected into CN in a much larger dose than in the case of old rats. It can accordingly be postulated that the presence of AB to L-dopa and DA in elderly but neurologically healthy persons is a factor of increased risk compared with the middle-aged group (Table 1), for the reliability of the blood-brain barrier (BBB) decreases with age [2], the DA level in the striatum falls [11], and the prevalence of parkinsonism increases [8]. Meanwhile, the increased content of AB to L-dopa and DA in patients treated with L-dopa may play a role in the formation of tolerance of patients to this preparation.

Immunologic "transfer" of Parkinson's syndrome from patients with parkinsonism to healthy animals by intracaudatal injection of AB to DA, isolated from patients, is similar to induction of the syndrome ln healthy rats after injection of rabbit GG, containing AB to DA, into their CN [5]. The fact that symptoms of parkinsonism also appear in rabbits immunized with DA suggests the possibility that AB to DA can pass from the blood into the brain through the BBB.

The appearance of manifestations of parkinsonism in some animals in response to injection of GG not containing AB to DA may be attributed to nonspecific traumatic irritation, and also to the presence of AB to neuroantigens (in particular, to the structures of CN), which are found in the blood of patients with parkinsonism [3, 6, 7]. It has been shown [12] that intraventricular injection of anticaudatal AB leads to the onset of hyperactivity of GPEE type in CN.

It can be tentatively suggested that AB to DA and AB to neuronal antigens have a pathogenic effect in the phase of progressive disease, which is characterized by disturbances of BBB and pathological changes in the state of DNSS. Under these conditions, AB to DA pass through the BBB and increase the DA deficit in CN, as result of which the cholinergic neurons of CN, normally inhibited by DA, undergo even further disinhibition, leading to potentiation and the development of a GPEE, resulting in further development of the syndrome.

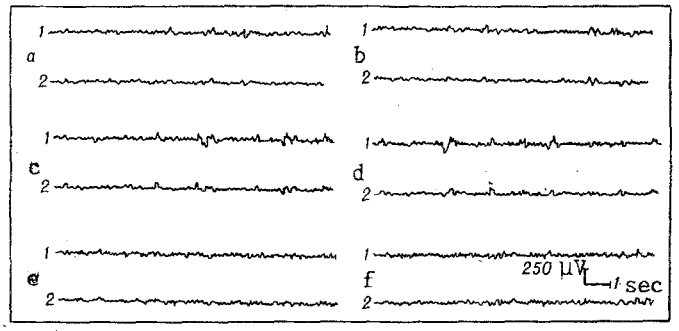

Fig. 2. Electrical activity in CN or rats after injection of GG, not containing AB to neurotransmitters, from patients with parkinsonism into nuclei. 1) Right CN, 2) left CN; a) before, b-f) after injection of GG: b) 5 min, c) 20 min, d) 40 min, e) 1 h 20 min, f) 3 h 50 min after injection.

Accumulation of AB to DA in the blood of healthy middle-aged and elderly persons deserves special attention. These AB may be a risk factor of CNS pathology, which is manifested when functions of BBB are disturbed. Under these conditions the AB can penetrate into brain tissue and inactivate DA, which can provoke or intensify neuropathological symptoms associated with insufficiency of the nigrostriatal mesolimbic or mesocortical dopaminergic system, depending on their predisposition to pathology and the locus of damage to BBB.

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