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EEG CHANGES AND MANIFESTATIONS OF PARKINSONISM FOLLOWING INTRACAUDATE INJECTION OF DOPAMINE ANTIBODIES

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The syndrome of parkinsonism has been shown [1, 3] to be connected with the formation of a generator of pathologically enhanced excitation (GPEE) [3] in the caudate nuclei (CN). GPEE formation may be the result of insufficiency of dopaminergic inhibitory control, leading to disinhibition of the cholinergic neurons of CN, or it may be an expression of primary hyperactivity of these neurons [1, 3, 4]. Insufficiency of the dopaminergic nigrostriatal system may be the result of injury to the dopaminergic neurons of the substantia nigra [15] or of a disturbance of dopamine (DA) secretion by the nerve endings of these neurons in CN [1, 3]. We know that antibodies to neurotransmitters can selectively bind the corresponding transmitters in the body and change the functional state of systems and organs [7, 10].

The aim of this investigation was to study the possibility of GPEE formation in CN and of thereby reproducing the basic symptoms of the syndrome of parkinsonism (oligokinesia, rigidity, tremor) by binding DA with antibodies in these nuclei.

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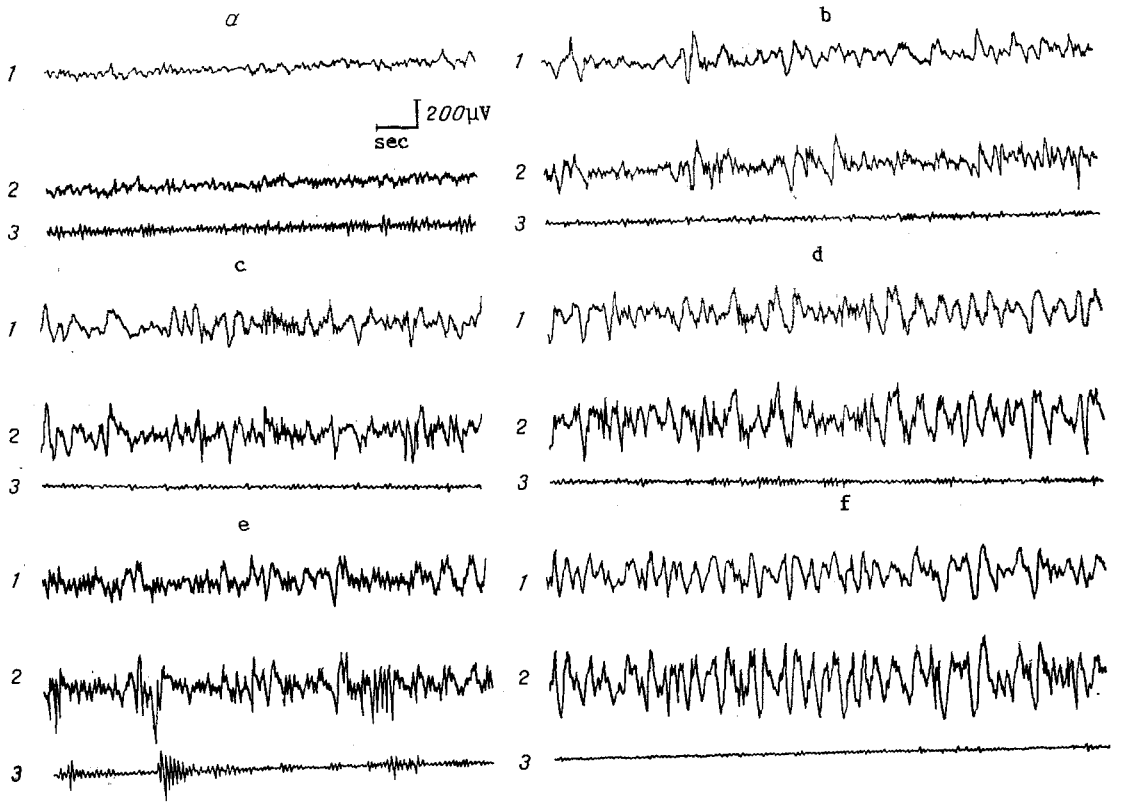

Fig. 1. EA in CN and actogram of animal after intracaudate injection of IG containing antibodies to DA. a) Background activity (episode of walking on actogram); b-f) 5, 10, 19, 21, and 47 min respectively after injection of antibodies to DA; 1) right CN, 2) left CN, 3) actogram (recording of tremor).

EXPERIMENTAL METHOD

Antibodies to DA were obtained by immunization of male chinchilla rabbits weighing 2.5-2.7 kg by a standard scheme with DA-protein conjugate. The DA-protein conjugate was synthesized by a modified method [13], using bovine serum albumin (BSA) as the protein carrier. The level of antibodies to DA was determined by ELISA, using a conjugate of DA and horse γ-globulin on a heterologous protein carrier as the test antigen. The level of antibodies to DA reached a maximum (1:2048) after immunization for 2.5 months. It was shown by ELISA that the antibodies bind free DA in vitro. γ-Globulin was isolated from sera of immunized and control animals by reprecipitation with ammonium sulfate [9]. γ-Globulin from the sera of two groups of animals was used as the control: control 1) rabbits immunized with carrier protein (BSA) by the same scheme; control 2) unimmunized rabbits, injected with physiological saline at the same times as for immunization. The γ-globulin was lyophilized and kept at 4°C. γ-Globulin isolated from the experimental and control sera and purified to remove antibodies to the carrier protein by affinity chromatography, using sepharose-4B, with BSA immobilized on it, as the immunosorbent, were used for intracerebral injection. Experiments were carried out on 29 male Wistar rats weighing 270-350 g. Under hexobarbital anesthesia, taking coordinates from a stereotaxic atlas [12], nicrome recording electrodes with a tip 0.2 mm in diameter were implanted into the rostral zones of CN bilaterally. The reference electrode was fixed in the nasal bones. The animals were kept in individual cages under standard animal house conditions on an ordinary diet. Immunoglobulins (IG), containing antibodies to DA were injected in a dose of 200 μg protein in a volume of 5 μl, at the rate of 1 μl/min, into each CN of rats of the experimental group 5-6 days after the operation. The intracerebral injections were given by means of a "Hamilton" microsyringe into a region located 1 mm from the recording electrode. Electrical activity (EA) in CN was recorded on an 8-channel polygraph (Nihon Kohden). EA was recorded continuously before injection of the antibodies, for 5-7 h after their injection, and for 1-2 h on the following day. The animals' motor activity was studied by the open field method, recording the number of squares crossed, the number of rearings on the hind limbs, the number of investigations of holes, and the num-

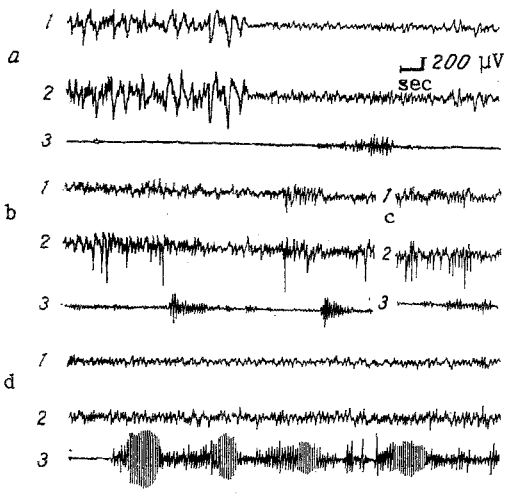

Fig. 2. EA in CN during manifestation of various forms of motor activity and tremor after intracaudate injection of antibodies to DA. a) After 2 h 32 min (episode of walking shown on actogram on right); b) after 22 h (short-term head tremor of average amplitude on actogram); c) after 22 h 30 min (low-amplitude head tremor on actogram); d) after 25 h (marked low-amplitude, high-frequency EA, coinciding with episode of grooming and scratching).

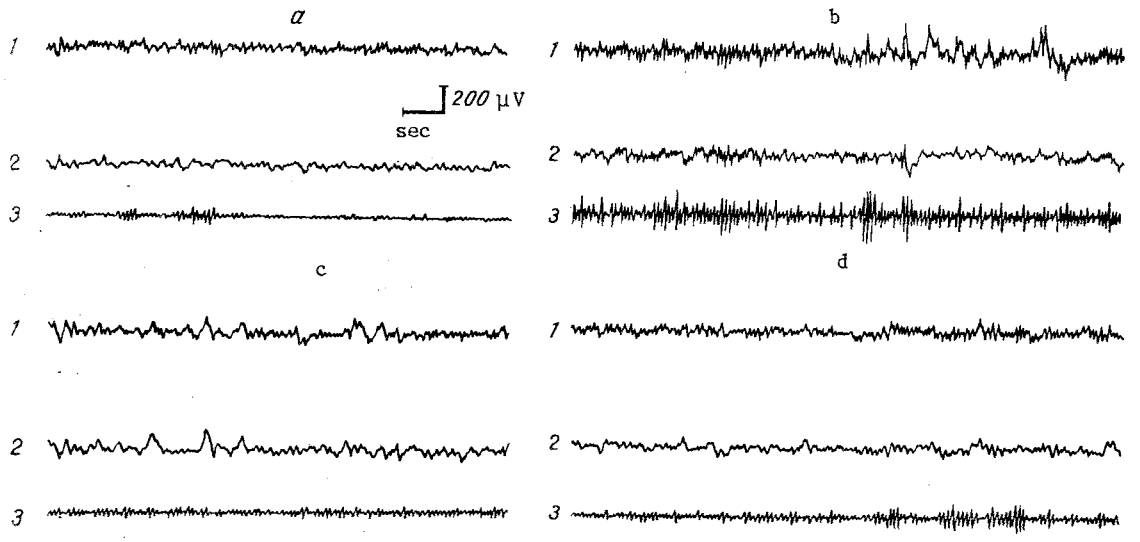

Fig. 3. EA in CN and actogram after intracaudate injection of γ-globulins from control serum 1. a) Background activity; b-d) after injection of γ-globulins: b) 5 min after (episode of walking on actogram), c) 10 min after, d) 19 min after (episode of walking on actogram).

ber of groomings in the course of 5 min. To assess muscular rigidity (MR), the "lordosis" sign was used; its severity depends on the degree of MR, and was determined as reduction in the distance from the neck to the base of the tail (the greater MR, the shorter this distance because of flexion of the animal's spine). Tremor was estimated visually and recorded by means of an actograph, constructed with the use of piezoelectric elements to record mechanical oscillations of low amplitude. Shaking of the animal's head, paws, and trunk was estimated from the frequency and amplitude of the waves recorded by the polygraph. The basic symptoms (oligokinesia, rigidity, and tremor) were assessed on a point system. Oligokinesia: +) reduction in the number of squares crossed and in other forms of motor activity; ++) reduction in the number of squares crossed and absence of other forms of motor activity; +++) absence of all forms of motor activity. Rigidity: +) shortening of the distance from the neck to the tail by 2 cm; ++) by more than 2 cm.

EXPERIMENTAL RESULTS

Spontaneous EA in CN was characterized by rhythm disturbances and the presence of low-amplitude fast and slow waves with an amplitude of 50-80 μV (Fig. 1a).

Single and grouped high-amplitude discharges with a frequency of 2-8/sec appeared in the EEG of all animals 6-8 min after injection of IG containing antibodies to DA (Fig. 1b). After 8-10 min periods of high-amplitude activity lasting from 27 to 66 min were recorded, with predominance of θ-waves with an amplitude of 600-700 μV (Fig. 1, c-f).

Injection of antibodies to DA into CN led to a marked decrease (++) of motor activity (oligokinesia). After 10-12 min, against this background of oligokinesia, short-term periods (1-2 min) of "freezing" were observed, with the appearance of sudden cessation of whatever behavioral act was in progress. After 17-21 min periodic akinesia (+++) appeared, and individual periods of this akinesia could last longer than 2 h (Fig. 1f). Periods of akinesia coincided with periods of high-amplitude activity, but they could be preserved during a period of low-amplitude activity in CN also. However, all froms of motor activity (walking, grooming, scratching, standing) were performed only against the background of low-amplitude activity with predominance of fast waves (Fig. 2a, d). Paroxysmal high-amplitude activity in CN and periods of hypokinesia and akinesia could be observed for 24 h. Similar observations were made [11] on a model of DA insufficiency induced in CN by 6-hydroxydopamine. In these experiments akinesia also correlated with hyperactivity of CN, whereas restoration of motor activity coincided with normalization of EA in CN. In the present experiments, six of 11 experimental rats developed rigidity (+ or ++). In nine rats, episodic low- and average-amplitude tremor of the head was observed (Fig. 1e) 8-20 min after injection of antibodies to DA, and in most cases this could last for 24 h (Fig. 2b, c).

In rats of control group 1, single and grouped waves with high (up to 300 μV) and average amplitude (up to 180 μV), with a frequency of 2-8/sec were found, and appeared 5-7 min after injection of the γ-globulins. These changes in EA were recorded for 7-10 min (Fig. 3b, c). Against this background oligokinesia (+) developed and lasted 30-40 min. In two of 13 rats, short-term low-amplitude head tremor was observed.

Injection of normal γ-globulin into CN (control 2) caused no significant changes in EA and did not lead to disturbances of the animals' motor activity.

Thus the results of the investigation are evidence of the formation of a GPEE in CN during binding of DA in these nuclei by antibodies, accompanied by the onset of oligokinesia and mild tremor and rigidity, i.e., manifestations of the syndrome of parkinsonism.

The results confirm the importance of insufficiency of the dopaminergic system in CN in the formation of GPEE and the role of GPEE as the pathogenetic mechanism of the syndrome of parkinsonism [3]. They are in agreement with the results of other investigations which have demonstrated the role of GPEE in CN in parkinsonism [1, 3, 4].

These investigations raise the question of the importance of DA antibodies in the development of parkinsonism. Antibodies to antigens of CN have been found in parkinsonism [2, 5, 6, 8, 14], but their importance in the pathogenesis of this disease has not yet been specially investigated. Nevertheless, the possibility that DA antibodies are involved in the development of parkinsonism has been demonstrated for the first time.

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DEPENDENCE OF HYPOXIC CHANGES IN MACRO- AND MICROCIRCULATION ON SKELETAL MUSCLE ADRENORECEPTOR ACTIVITY DURING HYPOTHERMIA

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It has been shown that hypoxia, superposed on hypothermia, causes changes in the macro- and microcirculation in skeletal muscle which differ qualitatively and quantitatively from changes taking place under the influence of a hypoxic stimulus during normothermia. The mechanisms of reduction of deviations or even reversal of the sign of parameters of the resistive and exchange (precapillary R_a- and postcapillary R_v-resistance, capillary filtration coefficient – CFC, and mean capillary hydrostatic pressure – P_m) functions of vessels of a skeletal muscle during exposure of an animal to hypoxic and hypothermic stimuli remain unchanged. According to data in the literature [5, 9] hypoxic hypoxia and acute hypothermia not only involve the direct action of oxygen deficiency in the blood and of cold on the contractile elements of the intramural vascular bed, but also cause various substances [13], including catecholamines [6], which together with other factors participate in changes in lumen of the arteries and veins, to enter the circulatory system. The intensity of the adrenergic component of the vascular changes during exposure to hypoxic and hypothermic stimuli differs: oxygen deficiency in the blood, if the duration of hypoxia is long enough, ultimately causes relaxation of vascular smooth muscles [12, 14], whereas hypothermia (to 30°C) causes their contraction [11] as a result of the action of catecholamines, the blood level of which rises considerably during cooling [6].

The aim of this investigation was to study the role of adrenergic mechanisms in changes in functional parameters of the vessels and transcapillary exchange of fluid in a skeletal muscle of an animal exposed to a combination of vascular factors induced by simultaneous hypoxia and hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on 22 cats of of both sexes (weighing 2.5-4.0 kg), anesthetized with urethane and chloralose (1.0 and 0.01 g/kg), receiving heparin (1500 U/kg). The gastrocnemius muscle (leg preparation) was isolated hemodynamically [2], the nerves were divided, and autologous perfusion was carried out with the animal's blood through the popliteal artery by means of a constant delivery pump [7]. Changes in R_a and R_v, P_m, and CFC were recorded by the method in [1]. Parameters of the macro- and microcirculation in the decentralized muscle were determined during hypoxia (inhalation of 10% oxygen in nitrogen, at the

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