AGE DIFFERENCES IN DEVELOPMENT OF A RESERPINE MODEL OF PARKINSONISM IN RATS

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The frequency of parkinsonism increases progressively with aging [3, 11]. To analyze the features of age which determine the development of parkinsonism, a model of this process in animals of different ages could be of great importance. In experimental practice a model has been used in rats in which the characteristic manifestations of parkinsonism developed as a result of chronic administration of reserpine [2, 5, 12]. However, this approach has not hitherto been used from the age aspect.

The aim of this investigation was to characterize the development of a reserpine model of parkinsonism in mature and old rats on the basis of data on the state of their motor activity and the electrical activity of certain structures of the extrapyramidal system.

EXPERIMENTAL METHOD

In experiments on nine mature (aged 9 months) and nine old (26 months) female rats monopolar nichrome electrodes (diameter of tip 0.15 mm) were implanted into the caudate nucleus (CN), globus pallidus (GP), and substantia nigra (SN), using data from a stereotaxic atlas [9]. A reference electrode, a nichrome wire coil, was inserted into the frontal bone at its boundary with the nasal bone. The spontaneous electrical activity of CN, GP, and SN was determined 21 days later, on unanesthetized, unrestrained, conscious animals, sitting quietly, on a type 4751-3 electroencephalograph with wide-band type 4657 EEG analyzer-integrator (Medicor, Hungary), recording the integral of EEG potentials during an epoch of 20 sec. The EEG recordings were analyzed automatically within the δ-, ϑ-, α-, β_1, β_2-, and γ-wave bands. Motor activity, the intensity of tremor, and the motor effects of electrical stimulation of CN (a 5-sec series of square pulses with a duration of 1 msec and a frequency of 10 Hz) also were evaluated. Motor activity was determined as the time of maintaining an enforced position of the body (EPB) – the time during which the rat stood on its hind limbs, supporting itself by its forelimbs on a horizontal bar. The intensity of tremor (in conventional units) was judged from the integral of the electrical waves in the 8-13 Hz band for 10 sec (the oscillations were generated by means of piezoelectric transducer in contact with the platform on which the animal stood, and led to the selector input of the EEG analyzer-integrator). The investigations were repeated 7 days later, during which time the animal was given daily intraperitoneal injections of reserpine solution in a dose of 1.5 mg/kg. At the end of the experiment the location of the electrode tips was identified. The significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

The results in Table 1 show that the initial values of EPB and tremor in the old rats were significantly higher and the threshold of the tremor-like motor response (shaking of the head and trunk in time with the frequency of stimulation) to electrical stimulation of CN was lower than in mature rats. Injection of reserpine for 7 days caused marked signs of parkinsonism in all the experimental animals: reduction of motor activity, increased tremor and lowering of the threhold of the tremor-like motor response. Changes in tremor in the old rats were significantly greater than in the mature animals, whereas a tendency toward a greater increase in EPB was discovered in the mature animals. Thus in old animals in the

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TABLE 1. Motor Activity, Tremor, and Minimal Strength of Stimulation Eliciting a Motor Response in Mature and Old Rats and Their Changes after Administration of Reserpine Daily for 7 Days

Parameters characterizing state of motor activity	Mature rats		Old rats	
	before injection of reserpine	after injection of reserpine	before injection of reserpine	after injection of reserpine
EPB, sec	4±1	+186±58*	13±4*	+56±19*
Tremor, conventional units	21±3	+61±7*	61±10	+172±43**
Threshold of motor response to stimulation of CN, μA	132±13	−73±10*	84±11*	−35±12**

Legend. Asterisk indicates significance of age differences ($P < 0.05$), cross indicates significance of shifts ($P < 0.05$).

TABLE 2. Spontaneous Electrical Activity of CN, SN, and GP in Mature (A) and Old (B) Rats

Parameter characterizing electrical activity (integral of EEG potential, V·sec)	CN		SN		GP	
	A (23)	B (26)	A (23)	B (27)	A (24)	B (27)
All waves	6,98±0,32	5,47±0,44**	6,59±0,34	5,42±0,42*	6,82±0,38	5,65±0,47
Separate waves						
δ	3,84±0,25	2,59±0,25***	3,88±0,26	2,98±0,27*	3,84±0,27	2,81±0,27**
ϑ	2,88±0,14	2,14±0,17**	2,72±0,17	1,87±0,11***	2,73±0,16	2,12±0,17*
α	2,22±0,11	1,80±0,15*	2,42±0,15	1,33±0,09***	2,18±0,16	1,79±0,15
β_1	1,34±0,06	1,04±0,10*	0,98±0,05	0,75±0,07*	1,29±0,08	1,13±0,11
β_2	0,62±0,03	0,46±0,05**	0,47±0,02	0,34±0,04**	0,68±0,04	0,58±0,06
γ	0,55±0,05	0,41±0,04*	0,38±0,03	0,31±0,04	0,42±0,04	0,38±0,04

Legend. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Numbers in parentheses are numbers of observations.

initial state signs of relative extrapyramidal insufficiency were found: reduction of motor activity expressed as an increase in EPB and intensification of tremor. In the old rats the threshold of the tremor-like response to stimulation of CN was depressed compared with mature rats.

Recently the neostriatum has been regarded as the subcortical level for complex sensomotor integration [6, 7]. In the light of data indicating that the source of tremor-like activity in parkinsonism is located in the basal ganglia [12], the age-associated lowering of the threshold of the compulsive tremor-like response to electrical stimulation of CN can be regarded as a manifestation of functional disintegration of the neostriatum.

The increase in EPB and intensification of tremor in the rats with age and the corresponding changes in these parameters after administration of reserpine are arguments in support of the similarity of the mechanisms of age-associated extrapyramidal insufficiency and the development of a reserpine model of parkinsonism.

An essential role in the development of parkinsonism has been shown to be played by disturbances of functional interaction between CN, SN, and GP [8]. Table 2 shows that electrical activity of these structures in rats does not change uniformly with age. For example, in old animals the integral of EEG potentials of all wave bands is significantly lower than in mature rats in CN, but only in the case of low-frequency wave bands is it lower in GP, in which, by contrast with the other structures, no age-associated slowing of δ-waves was recorded.

As a result of daily administration of reserpine for 7 days 55% of the mature and 44% of the old rats died. Death occurred at the end of the 7-day administration of reserpine and between the 9th and 21st days after the beginning of its injection. Recording electrical activity of the nuclei of the extrapyramidal system under chronic experimental conditions enabled these changes to be compared with the survival rate of the animals. The development

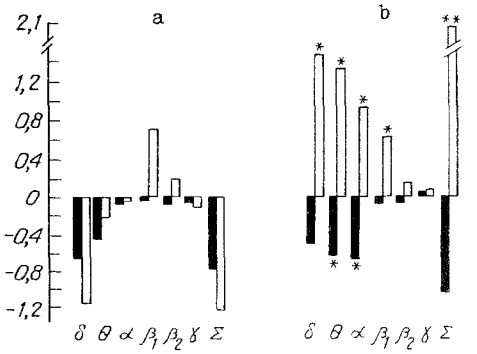

Fig. 1. Changes in integral of EEG potentials of all waves (Σ) and of individual waves of CN after administration of reserpine daily for 7 days to mature (a) and old (b) rats. Ordinate changes in integral of EEG potentials (in V·sec). Unshaded columns – surviving rats, black columns – dying rats. Cross indicates significance of shift ($p < 0.05$); asterisk indicates significance of age differences in corresponding groups of animals ($p < 0.05$).

of reserpine-induced parkinsonism in the old rats was accompanied by more marked changes in the integral of EEG potentials of nuclei of the extrapyramidal system compared with mature rats. Qualitative age differences also were found in the shifts of electrical activity. Changes in the integral of potentials of CN in mature and old rats 24 h after the last (seventh) injection of reserpine are illustrated in Fig. 1. In the old rats which subsequently survived during the experiment, a statistically significant increase or a tendency toward an increase was found, whereas in rats which subsequently died, the integral of the potentials decreased. Meanwhile, in the mature rats after administration of reserpine for 7 days a tendency was found for high-voltage low-frequency waves to decrease and for the integral of potentials of the β_1-waves to increase. Definite correlation also was found between the character of the percentage distribution of the integral of potentials of individual waves in the spectrum of the initial EEG traces obtained from CN in old rats and their mortality due to chronic reserpine poisoning. For instance, in the subgroup of rats which died the fraction of ϑ-waves on the initial EEG traces from CN was 30 ± 0.9%, compared with 24 ± 0.4% in the subgroup which survived ($p < 0.001$). The corresponding values for the β_2-waves were 3.7 ± 0.9 and 6.0 ± 0.4% ($p < 0.05$). The ratio between the parameters of the distribution of individual waves in the spectrum of EEG recordings from CN in the subgroups of mature rats which died and survived did not differ significantly from each other. Thus with age, the relationship between electrical activity of CN and the consequences of chronic reserpinization is strengthened in rats.

Thus signs of functional insufficiency of the extrapyramidal system appear in rats during aging. Rats are not prone to develop spontaneous atherosclerosis, so that an atherosclerotic process can be eliminated from the list of factors responsible for the development of this age-induced extrapyramidal insufficiency. An important role in its development may perhaps be played by changes in functional interaction between CN, GP, and SN, evidence for which is given by differences in age changes in their electrogenesis. Functional discoordination between structures of the extrapyramidal system may be linked with a deficiency of nigro-striatal dopamine [8]. During aging the dopamine concentration in the striatum falls [10]. Dopamine has a mainly inhibitory action on striatal neurons [12, 13], and for that reason hyperactivation of certain elements of CN may arise during aging, just as during long-term reserpinization. The development of experimental parkinsonism has been shown to be accompanied by the formation of a generator of pathologically enhanced excitation in CN [1]. Evidence of the possibility that activity of CN may be enhanced in this way during aging and during long-term reserpinization in the present experiments is given by lowering of the threshold of the tremor-like response to electrical stimulation of CN and the increase in its spontaneous electrical activity in some animals during the development of experimental parkinsonism.

The development of reserpine-induced parkinsonism in the mature rats was manifested mainly as limitation of mobility, whereas in the old rats it was manifested as intensification of tremor. Under these circumstances the fact will be noted that in patients aged under 40 years an akineticorigid form of parkinsonism is most frequently observed, whereas in older patients it is the tremoro-rigid form [4]. The existence of neurodynamic differences in the development of the reserpine model of parkinsonism in different age periods is shown by the absence of a uniform trend of the changes in electrical activity of the extrapyramidal nuclei studied in animals receiving long-term reserpine therapy.

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