CHARACTERISTICS OF THE BRAIN DOPAMINE SYSTEM IN MICE WITH THE NEUROLOGICAL QUAKING MUTATION

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The autosomal recessive quaking mutation in mice disturbs differentiation of oligodendrocytes, which leads to demyelination of the CNS [13, 14]. The behavioral manifestation of its mutation, which is controlled by a single gene, consists of constant tremor of the limbs and trunk, abnormal locomotion, and tonic convulsions, which may arise spontaneously. The effects of the mutation are completely manifested in mice at the age of about 1 month, and only in animals carrying the quaking gene in the homozygous state; heterozygotes are phenotypically normal. Since one of the basic features of quaking mice is the disturbance of locomotion, essential changes can be postulated in the dopamine (DA) system of the brain, which is involved in the regulation of motor activity and stereotyped behavior (see the surveys [1, 3]), but no data on the state of the brain DA system of these animals could be found in the literature.

The aim of the investigation was to study DA metabolism and the functional state of the receptor mechanisms of the brain DA system in mice with the neurological quaking mutation.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male mice aged 3-4 months and weighing 20-25 g, carrying out quaking (qk) mutation in the homozygous (qkqk) and heterozygous (qk+) state. Phenotypically normal heterozygotes, in which the quaking gene does not exhibit its action, were used as the control. The mice were kept under standard animal house conditions, four to a cage during the testing period, and 2 days before decapitation the mice were put into individual cages in order to abolish group-dependent influences. Spontaneous motor activity was recorded in an Animex apparatus for 20 min and in the open field test, the number of squares crossed during 5 min of testing being counted. The effect of administration of apomorphine was assembled similarly.

Stereotyped climbing behavior was observed in individual cylindrical wire mesh cages 12 cm in diameter and 14 cm high 10 min after administration of the test preparations, by recording climbing every minute for 20 min [10]. The following scale was used to evaluate climbing: 0) mouse on the cage floor, 1, 2, 3, 4) mouse holding the vertical wall of the cage with one, two, three, or four limbs. The number of points during the period of observation was added together.

The following drugs were used to act upon the brain DA system: apomorphine hydrochloride (Sigma) in a dose of 0.25 mg/kg, affecting predominantly presynaptic DA receptors [5], and in a dose of 2.5 mg/kg, stimulating postsynaptic DA receptors [12]; haloperidol (Reanal) in a dose of 0.1 mg/kg; SKF-38393 (Smith, Kline, and French) in a dose of 10 mg/kg. The preparations were dissolved in distilled water immediately before the injection and were injected in a dose of 0.1 ml of solution per 10 g body weight. Groups of 6-8 animals were used for pharmacological analysis.

Mice of another group were decapitated, the brain was removed in the cold, and the corpus striatum and nucleus accumbenes were separated together with the olfactory tubercles. DA and its metabolites — 3,4-dihydroxyphenylacetic acid (DOPAA) and homovanillic acid (HVA) — were determined fluorometrically after chromatographic fractionation on Sephadex F-10 [4].

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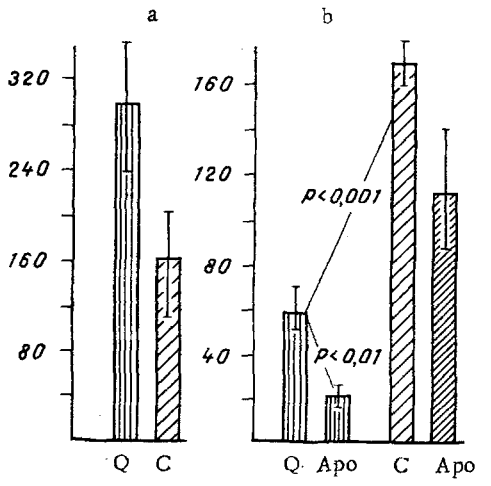

Fig. 1

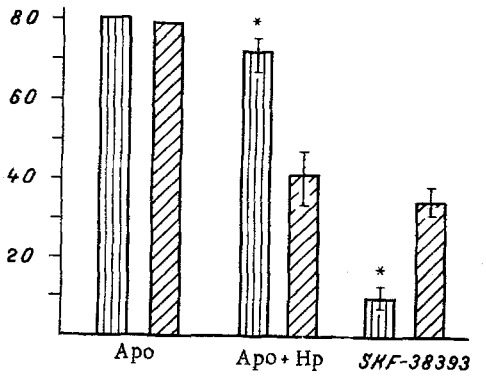

Fig. 2

Fig. 1. Motor activity of quaking mice (Q) compared with control mice (C), recorded in Animex (a) and open field (b). Apo) Effect of injection of apomorphine, 0.25 mg/kg. Ordinate, number of motor acts.

Fig. 2. Manifestation of climbing (in points -- ordinate) by quaking mice (vertical shading) compared with control (oblique shading). Apomorphine (Apo) injected in a dose of 2.5 mg/kg. haloperidol (Hp) in a dose of 0.1 mg/kg, and SKF-38393 in a dose of 10 mg/kg. Asterisk indicates significant ($p < 0.05$) difference between groups.

The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The study of spontaneous motor activity in the Animex apparatus showed that locomotor activity of quaking mice is somewhat greater than that of control animals. However, since the instrument records both moving about and tremor, the locomotion of the quaking mice was assessed by the open field test. The number of squares crossed by mice with the neurological mutation in the open field was almost 3 times less than the number of crossed by control animals, and it will be evident that the number of effective motor reactions of the mice with the quaking mutation was significantly less than that of phenotypically normal heterozygotes. Injection of apomorphine in a dose of 0.25 mg/kg, stimulating mainly presynaptic DA receptors [5], reduced motor activity of both groups of mice in the open field. However, significant inhibition of locomotion was observed only in quaking mice, a result which evidently reflects the greater sensitivity of presynaptic DA-receptor in mice with hereditary demyelination (Fig. 1).

Injection of the large dose of apomorphine (2.5 mg/kg) led to the appearance of stereotypes behavior (climbing), manifested by continuous climbing by the mouse up to vertical wall of the cage. Climbing was expressed to the greatest degree in both groups of mice (Fig. 2). However, injection of haloperidol, a DA-receptor antagonist, in a dose of 0.1 mg/kg blocked the manifestation of stereotypes climbing much less in the quaking mice than in the control group. Since haloperidol inhibits postsynaptic DA-receptors, on which apomorphine acts in the dose given [7], it will be evident that the sensitivity of the postsynaptic DA-receptors of the quaking mice is less than that of the controls.

DA-receptors are subdivided into two types: type 1 (D_1), bound with adenylate cyclase, and type 2 (D_2), not so bound [6]. Apomorphine, a mixed DA-receptor antagonist, possesses great affinity for D_2 receptors and weak affinity for D_1 receptors [12]. Analysis of stereotypes behavior by the use of the selective D_1-receptor agonist SKF-38393 [10] showed that it induces climbing in both groups of mice, but its action was much weaker on the climbing mice, evidence that their D_1 receptors are less sensitive or less numerous. Comparison of the action of apomorphine and SKF-38393 on the manifestation of climbing suggests that homozygotes for the quaking mutation have similar sensitivity of their D_2 receptors with the control animals, but they differ in the state of their D_1 receptors.

TABLE 1. Concentrations of DA, DOPAA, and HVA (in μg/g tissue) in Brain Structures of Mice Carrying the Quaking Mutation (M ± m)

Brain structure	Group of animals	DA	DOPAA	HVA
Corpus striatum	Quaking (n = 8)	8,821±1,07	1,96±0,19	0,77±0,09**
	Control (n = 8)	8,42±0,59	1,41±0,20	0,37±0,08
Nucleus accumbens with olfactory tubercles	Quaking (n = 8)	3,47±0,39	0,79±0,05*	0,31±0,03
	Control (n = 8)	3,69±0,38	0,63±0,04	0,24±0,07

Legend. *p < 0.05; **p < 0.01 compared with control; n) number of experiments.

Determination of DA metabolism in the corpus striatum and nucleus accumbens with olfactory tubercles revealed no differences in the level of the mediator of the structures tested in quaking mice compared with the control (Table 1). Meanwhile, the concentrations of metabolites were higher in the quaking mice: the HVA level in the corpus striatum and the DOPAA level in the nucleus accumbens of these mutants were significantly higher than in the control. This is evidence of increased DA turnover in quaking mice, both in the nigrostriatal dopamine system and in the mesolimbic system. It must be pointed out that in quaking mice the corpus striatum contained more HVA — a net product of DA catabolism formed by O-methylation and oxidative deamination. In the nucleus accumbens oxidative deamination takes place more activity with increased formation of DOPAA.

Quaking mice, characterized by depressed locomotor activity and tremor, thus have modified sensitivity of their synaptic DA receptors, reduced sensitivity of D_1 receptors, and increased DA turnover in structures of the mesolimbic and nigrostriatal DA system. It must be emphasized that the principal phenotypic manifestation of the quaking mutation, namely abnormal locomotion, is accompanied by changes in the brain DA system controlling locomotor activity and the onset of stereotypy. Investigation of the noradrenalin system of the brain in quaking mice revealed enhanced noradrenergic transmission: an increased concentration of noradrenalin and its metabolite, an increase in the number of noradrenergic neurons, and altered sensitivity of adrenoreceptors, with which the manifestation of tonic convulsions in these animals had been tentatively linked [2, 9]. On the basis of our own observations and the results of the study of the noradrenergic system [2, 9], it can be postulated that the altered state of the brain catecholamine system may be a linked effect of the quaking mutation and not a simple consequence of the disturbance of myelination. The enzyme cyclic adenosine-2',3'-phosphate 3'-nucleotide hydrolase is absent in quaking mice [8], and the deficiency of this enzyme evidently has a pleiotropic action on the catecholamine systems. The changes discovered in different stages of the brain DA system of quaking mice indicate possible points of application for correction of the pathological states arising in demyelinating disturbances.

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ROLE OF *Escherichia coli* HOST CELLS IN GENETIC CONTROL OF PLASMID TRANSFER

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The genomes of different plasmids contain the genetic fin-systems OP, Q, U, V, W, and C, which control the synthesis of inhibitors of transfer of F and other F-like plasmids [5, 8]. The use of derepressed plasmids with known type of sensitivity toward transfer inhibitors, suppressing the action of tra-genes, has led to the identification of the genetic fin system in a number of repressed plasmids [1]. However, the regulating activity of these systems in all known experiments has been established by the use only of cells of a serologically untyped laboratory strain *E. coli* K12 and its derivatives. Yet under natural conditions plasmids are inhabitations of cells of *E. coli* strains which differ significantly from the K12 strain, thus raising the question of the role of host cells in the genetic control of plasmid transfer.

The aim of the investigation was to study genetic control of plasmid transfer in serologically typed *E. coli* cells.

EXPERIMENTAL METHOD

Strains *E. coli* C600 Str and AP132 Nal, derivatives of *E. coli* K12 forming rough colonies (the R form) were used. Genetically labeled strains of serologically types *E. coli* forming smooth colonies (S) were bred in the course of the present investigation. The S and R forms of the bacteria were differentiated in tests with boiling of the corresponding cultures. O antigens were detected in the linear agglutination test in tubes, using heated cultures of bacteria and standard OB-coli diagnostic sera.

R plasmids pAP3 (Lm), pAP17-2 (Tc), pAP18-1 (Tc), pAP30-2 (Ap, Lm, Tc), and Hly-plasmid pAP17-1::Tn9, controlling synthesis of known types of inhibitors [1], were used as plasmids repressed for transfer functions. The list of derepressed plasmids with known types of sensitivity to inhibitors used is given in Table 1. Plasmids were eliminated from bacteria with the aid of ethidium bromide by the standard method [7]. Conjugation transfer of plasmids was carried out in standard crosses of plasmid donor bacteria with suitable recipient cells [3].

The ability of *E. coli* cells to form specific F-pili was judged by their sensitivity to F-pili-specific phage MS2, determined by the agar layers method [3]. The relative seeding efficiency of the phage was determined as the ratio between the mean number of infectious phage centers, formed after seeding biplasmid conjugants (cells containing a derepressed and a repressed plasmid at the same time) to the number of such centers formed in the case of monoplasmid bacteria (containing one derepressed plasmid only), in percent. The transfer inhibition index of the derepressed plasmid was determined as the ratio of the frequency of conjugation transmission of this plasmid from cells of the monoplasmid strain to the corresponding parameter for the biplasmid strain.

EXPERIMENTAL RESULTS

The investigation began with the obtaining of genetically labeled recipient (plasmid-free) strains of serologically types *E. coli*. For this purpose, after treatment of the plasmid-containing cells of *E. coli* of strains AP15 (serogroup O106), AP58 (O147), and AP70 (O128), isolated previously [2, 4], with ethidium bromide, plasmid-free variants of these

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