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Correlation Between Tryptophan Hydroxylase Activity in the Brain and Predisposition to Pinch-Induced Catalepsy in Mice

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KULIKOV, A. V., E. Y. KOZLACHKOVA, N. N. KUDRYAVTSEVA AND N. K. POPOVA. *Correlation between tryptophan hydroxylase activity in the brain and predisposition to pinch-induced catalepsy in mice.* PHARMACOL BIOCHEM BEHAV **50**(3) 431–435, 1995.—Pinch-induced catalepsy and activity of the rate-limiting enzyme of serotonin biosynthesis, tryptophan hydroxylase, in brain structures have been studied in mice of six inbred strains. A pronounced predisposition to catalepsy was found in the CBA mouse strain. It was shown that the CBA mice had the highest tryptophan hydroxylase activity in the neostriatum compared to mice of other noncataleptic strains. The experience of repeated victories in intermale encounters producing highly aggressive CBA mice resulted in the inhibition of the genetically determined predisposition to pinch-induced catalepsy and in the simultaneous decrease of tryptophan hydroxylase activity in the neostriatum down to the level found in noncataleptic mice. The inhibitor of tryptophan hydroxylase, *p*-chloromethamphetamine, significantly decreased the enzyme activity in the neostriatum of CBA mice and completely inhibited their genetically determined predisposition to catalepsy. These findings indicate the key role of the striatal serotonergic system in the catalepsy-inducing mechanism.

Inbred mice Catalepsy Tryptophan hydroxylase Neostriatum

CATALEPSY (tonic immobility, immobility reflex, animal hypnosis) is a state of pronounced movement inhibition, characterized by a failure to correct an externally imposed, awkward posture. This phenomenon represents an element of defensive behavior and is found in all classes of vertebrates (i.e., fishes, amphibians, reptiles, birds, and mammals) (11). It is believed that catalepsy is associated with fear and manifested as freezing in response to the appearance of a predator or other threatening stimulus (8). This reaction was also suggested to be an element of male submissive behavior (6). In an exaggerated form, catalepsy is a syndrome of Parkinson's disease (5), schizophrenia (9,25), and Huntington's disease (24).

There is evidence for the involvement of brain serotonin in the development of catalepsy (8,12,13,18,21). A relationship between activity of the rate-limiting enzyme of serotonin biosynthesis tryptophan hydroxylase and predisposition to catalepsy in rats was found. Rats of a strain bred for predisposition to catalepsy had increased tryptophan hydroxylase activity in the neostriatum compared to nonselected, random-bred Wistar rats (13). Treatment of the cataleptic rats with a tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine, decreased the immobility time (18). It was hypothesized (13,21) that the striatal serotonergic system played an essential role in the mechanism of hereditary catalepsy.

Inbred mice give good opportunity for studying the genetic and functional correlations between tryptophan hydroxylase activity and catalepsy. Firstly, substantial interstrain differences in predisposition to pinch-induced catalepsy (17) and in tryptophan hydroxylase activity in the brain of inbred mice (19) have been found. Secondly, male mice with consecutive experience of repeated victories in intermale agonistic confrontations never displayed freezing (15,16). Thirdly, *p*-chloromethamphetamine was found to inhibit tryptophan hydroxylase activity in mouse brain (10).

In the present work the following specific aims were intended to be accomplish: 1) to estimate tryptophan hydroxylase activity in the brain of mice with hereditary differences in the predisposition to pinch-induced catalepsy; 2) to compare the predisposition to catalepsy and the enzyme activity in the

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brain of mice with consecutive experience of repeated victories or defeats in intermale confrontations; 3) to study the effect of *p*-chloromethamphetamine on hereditary catalepsy in mice.

METHOD

Animals

Adult male mice (2–3 months of age, weighing 20–30 g) of six inbred strains (AKR, BALB/c, CBA/Icg, CC57Br, C57BL/6, and DD), maintained at the Institute of Cytology and Genetics, Novosibirsk, by close inbreeding for 20 years, were used. The animals were housed in groups of 10 per cage and kept under controlled environmental conditions (room temperature 22°C; standard chow and tap water ad lib). A day before the experiments, animals were isolated in steel individual cages (28 × 14 × 10 cm) to assess the baseline level of the neurochemical parameters and predisposition to catalepsy.

Induction of Submissive and Aggressive Behavior

A pair of CBA mice of the same weight was placed into a steel cage (28 × 14 × 10 cm) separated into two halves by a transparent partition with holes permitting the animals to see and smell each other but preventing them from physical contact. After 2 days of adaptation to the housing conditions and sensory contact, the testing was started: every afternoon the steel cover of the cage was replaced by a transparent one, and 5 min later the partition was removed for 10 min, which led to agonistic interaction between the males. Clear superiority of one of the partners was evident within two to three tests in daily social encounters in the same cage. One partner demonstrated aggression, attacking, biting, and chasing the other one, which displayed defensive behavior (sideways, upright postures, or freezing). After 15 days of training, one mouse of each pair had a consistent history of victory (winner), whereas the other had a history of defeats (loser). Untrained mice were used as controls.

Drug

The selective tryptophan hydroxylase inhibitor (10) *p*-chloromethamphetamine (Serva, Germany) was dissolved in saline and administered (15 mg/kg, IP) to cataleptic CBA mice. Effect of *p*-chloromethamphetamine on immobility time and on tryptophan hydroxylase activity was tested 3 h after the administration.

Catalepsy Test

The method used to elicit pinch-induced catalepsy did not differ from the original method of Amir et al. (1,20). An animal was firmly pinched between two fingers for 5 s at the scruff of the neck. It was then placed on parallel bars, with the front paws situated at a 45° angle above the hind paws, and released gently. The time of freezing was recorded. The freezing was considered as catalepsy when its time was 20 s or more. Each test was limited to 2 min. Every animal was submitted to 10 successive tests with 2-min intervals. A mouse was given a "cataleptic" score according to the number of positive tests for catalepsy out of 10. The animal was considered as cataleptic if it demonstrated catalepsy in at least three cases. Predisposition to catalepsy was evaluated by the percentage of cataleptic mice in the strain.

After testing, animals were decapitated; their brains were removed and chilled rapidly on ice. The neostriatum, hippocampus, and midbrain were isolated, rapidly frozen, and stored at −70°C until use but not longer than a week.

Tryptophan Hydroxylase Assay

The samples were homogenized with 5 vol. of 50 mM Tris acetate buffer (pH 7.5) containing 1 mM dithiothreitol (Sigma). The homogenates were centrifuged at 18,000 × *g* for 30 min (+4°C). The enzyme activity was assayed in the supernatant in the presence of *l*-tryptophan (0.8 mM, Sigma, St. Louis, MO) and 6,7-dimethyl-5,6,7,8-tetrahydropteridine (0.5 mM, Sigma) by a fluorescence microassay described in detail elsewhere (18) and expressed in pmol of 5-hydroxytryptophan formed per mg of protein per min.

Statistics

Interstrain differences in percentage of cataleptic mice were evaluated by chi-square test (6), and those in brain tryptophan hydroxylase activity were tested by ANOVA followed by post hoc Scheffe's S-multiple comparisons using STATGRAF/PC set of programs. Comparison between two groups of data was made by Student's *t*-test.

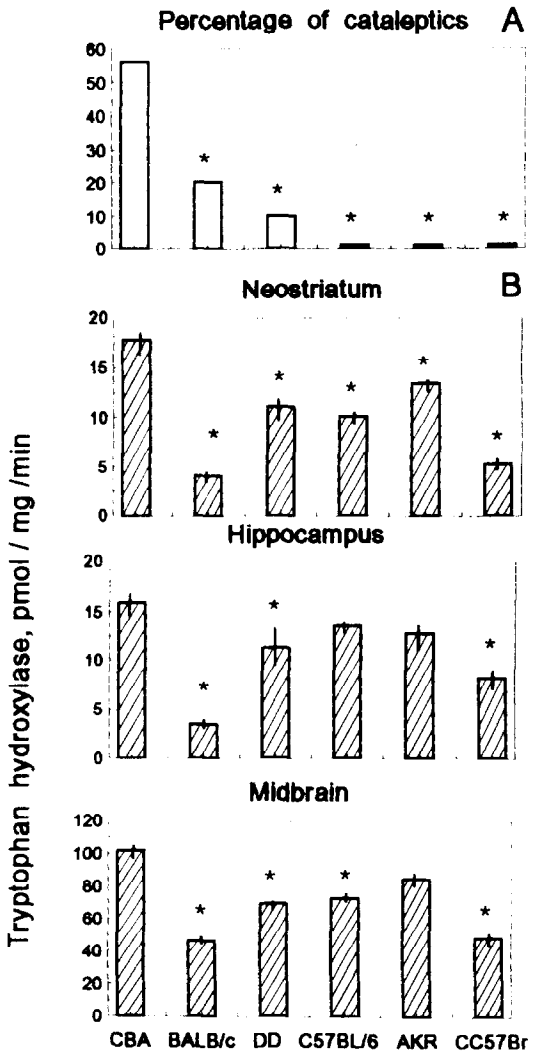

FIG. 1. Percentage of cataleptics (A) and the tryptophan hydroxylase activity in the brain (B) in male mice of inbred strains. Bars represent the means ± SEM at least of seven determinations in the neostriatum and hippocampus, and six determinations in the midbrain. *$p < 0.05$ vs. CBA mice.

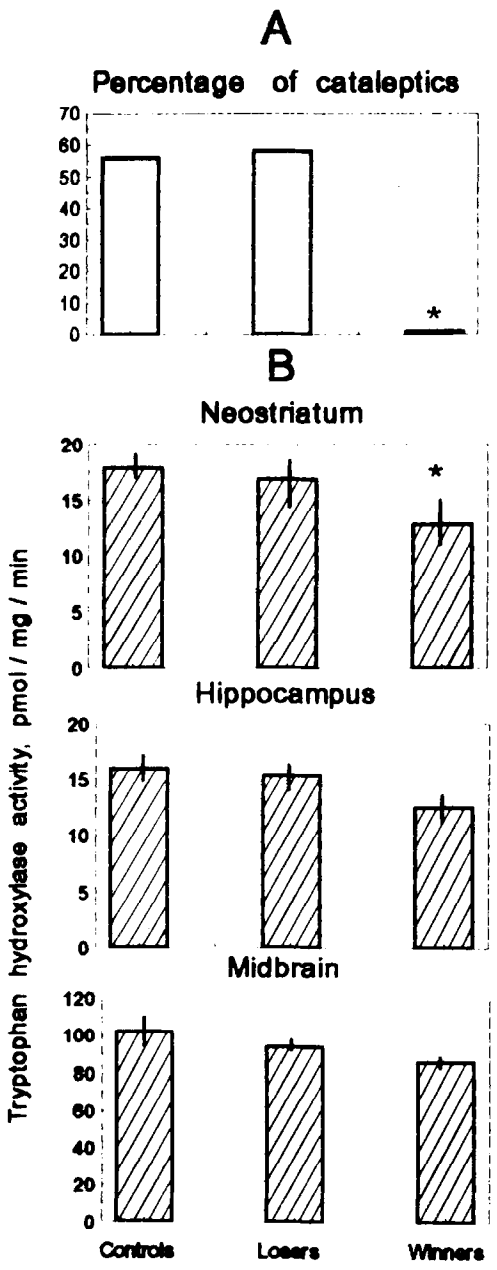

FIG. 2. Percentage of cataleptics (A) and the tryptophan hydroxylase activity in the brain (B) in CBA mice with a consistent history of victory (winners) or defeats (losers) in daily intermale agonistic conflicts, or untrained mice (control). Bars represent the means ± SEM at least of eight determinations. *$p < 0.05$ vs. controls.

RESULTS

Essential interstrain differences in the percentage of animals displaying catalepsy were found, $\chi^2(5) = 32.6, p < 0.01$ (Fig. 1A). Mice of three strains (AKR, C57BL, and CC57Br) never demonstrated catalepsy. Catalepsy was found in one DD and two BALB/c mice out of 10 tested. At the same time, in CBA strain freezing exceeding 120 s was developed after four to five tests in 15 out of 27 mice (56%).

There were significant interstrain differences in the enzyme activity found in the neostriatum, $F(5, 36) = 49.1$, $p < 0.001$, hippocampus, $F(5, 36) = 23.4$, $p < 0.001$, and midbrain, $F(5, 33) = 18.8$, $p < 0.001$ (Fig. 1B). Tryptophan hydroxylase activity in these brain structures in BALB/c and CC57Br mice were lower than in CBA, AKR, C57BL, and DD mice. The highest tryptophan hydroxylase activity was found in the brain of mice of the cataleptic CBA strain compared to mice of other strains. The most pronounced differences were revealed in the neostriatum: the enzyme activity in this structure in CBA mice was significantly higher than in mice of any other strains tested ($p < 0.05$). In the midbrain and hippocampus these differences were less marked. Tryptophan hydroxylase activity in these structures in cataleptic CBA mice did not differ from those in noncataleptic AKR mice ($p > 0.05$), though it was higher than in mice of other noncataleptic strains.

Seven out of 12 (58%) CBA mice with consecutive experience of repeated defeats in daily intermale agonistic confrontations displayed the pinch-induced catalepsy similarly to control CBA mice, $\chi^2(1) = 0.04$, $p > 0.05$. However, pinching the skin at the scruff of the neck did not induce any cataleptic-like freezing in all 12 CBA mice with experience of repeated victories ($p < 0.0007$) (Fig. 2A). At the same time, the enzyme activity in the neostriatum of the winners was significantly lower than in that structure of controls, $t(18) = 2.5$, $p < 0.05$. No differences in tryptophan hydroxylase activities in the midbrain and hippocampus were found between controls, losers, and winners (Fig. 2B).

For further investigation of the association between the predisposition to catalepsy in CBA mice and the increased serotonin biosynthesis in the neostriatum, the effects of *p*-chloromethamphetamine on tryptophan hydroxylase activity and duration of freezing were studied (Fig. 3). *p*-Chloromethamphetamine (15 mg/kg, IP) was shown to inhibit the catalepsy. None of the 32 cataleptic mice showed any freezing 3 h after an injection of *p*-chloromethamphetamine, $t(31) = 11.1$, $p < 0.001$. The inhibition of freezing was associated with a pronounced decrease in tryptophan hydroxylase activity in the neostriatum of *p*-chloromethamphetamine-treated mice, $t(10) = 2.3$, $p < 0.05$ (Fig.3). An injection of saline did not affect immobility in any of 15 cataleptic CBA mice, $t(14) = 0.31$, $p > 0.05$.

DISCUSSION

It has been shown that mice of the CBA strain had genetically determined predisposition to pinch-induced catalepsy and a higher tryptophan hydroxylase activity in the neostriatum compared to mice of the other strains tested. These data strongly suggest that the genetic factor determining the in-

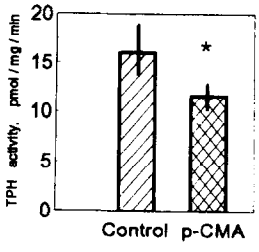

FIG. 3. Effect of *p*-chloromethamphetamine (*p*-CMA, 15 mg/kg, IP, 3 h) on tryptophan hydroxylase activity in the neostriatum of CBA mice. Bars represent the means ± SEM of six determinations. *$p <$ 0.05 vs. saline-treated mice.

crease in the enzyme activity in the neostriatum is involved in the mechanism of inherited predisposition to catalepsy. It is noteworthy that the activation of the rate-limiting enzyme of serotonin biosynthesis was shown to reflect the increased serotoninergic neuron firing rate and serotonin release (4). The lack of any genotypic correlation between predisposition to catalepsy and tryptophan hydroxylase activity in the midbrain, where the enzyme is synthesized, suggests that catalepsy in mice is not produced by an alteration in the structure or expression of the gene encoding tryptophan hydroxylase. It may be suggested that the activation of tryptophan hydroxylase in the neostriatum of CBA mice results from a reversible phosphorylation of the enzyme, as it has been earlier reported for rats with genetically determined predisposition to catalepsy (18).

The lack of cataleptic-like immobility in the aggressive CBA mice is consistent with the notion that aggression and freezing represent two opposite kinds of defensive behavior in intermale conflicts (3). This inhibition of predisposition to pinch-induced catalepsy in the winners is associated with a significant decrease in tryptophan hydroxylase activity in their neostriatum, but not in the hippocampus and midbrain, down to the level of the enzyme activity found in mice of a noncataleptic AKR strain.

The tryptophan hydroxylase inhibitor, *p*-chloromethamphetamine, drastically decreased tryptophan hydroxylase activity in the neostriatum of CBA mice. These alterations in the enzyme activity were accompanied by a complete inhibition of the predisposition to catalepsy in the drug-treated mice.

Therefore, three independent lines of evidence mentioned above indicate genetic and functional relationships between the striatal tryptophan hydroxylase activity and predisposition to catalepsy in mice: (i) the enzyme activity in the neostriatum in mice of cataleptic CBA strain is higher than in the neostriatum in mice of any noncataleptic strain; (ii) winner mice have lower tryptophan hydroxylase activity in the neostriatum and do not display catalepsy; (iii) the inhibition of tryptophan hydroxylase prevented expression of catalepsy.

Earlier we found that breeding the rats for predisposition to catalepsy was also accompanied by a selective increase of tryptophan hydroxylase activity in the neostriatum due to local phosphorylation of the enzyme (13,18,21). It was shown that the catalepsy in Wistar rats produced by a chronic methylphenidate administration or by audiogenic seizures was also associated with a selective tryptophan hydroxylase activation in this brain structure (13). Inhibition of the enzyme with *p*-chlorophenylalanine was shown to decrease the expression of catalepsy in cataleptic rats (18). It was suggested that the activation of tryptophan hydroxylase in the neostriatum was a necessary condition of the catalepsy development in rats. The consistent increase in tryptophan hydroxylase activity observed in cataleptics in two different species, rats and mice, suggests a similarity of genetic mechanisms that determine the predisposition to catalepsy and provides further evidence supporting our idea (21) that the striatal serotonergic system is involved in the crucial mechanism of catalepsy.

It is commonly accepted that catalepsy results from the deficiency of dopaminergic transmission in the neostriatum (12,23). Contrary to this theoretical scheme, the present data are consistent to some extent with the oppositional model of the serotonergic-dopaminergic interaction in the neostriatum; that is, the catalepsy may be produced by activation of the striatal serotonergic system as well as inhibition of the dopaminergic system (20). Dopamine was shown to inhibit serotonin release in the substantia nigra (2), and a blockade of the dopaminergic system inducing catalepsy seems to increase serotonin release. The inhibitory effect of *p*-chlorophenylalanine on the expression of hereditary and haloperidol-induced catalepsy (14,18) indicates that the serotonergic system is a major factor contributing to pathophysiology of catalepsy.

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