Epileptiform Activity in the Hippocampus of Mice with Different Predisposition to Pinch Catalepsy

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Experiments on hippocampal slices of CBA mice showed that the probability of spontaneous epileptiform discharges recorded in the field CA1 pyramidal layer is higher in animals predisposed to catalepsy compared to mice with low predisposition to catalepsy. Presumably, some factors determining predisposition to catalepsy modulate synchronization of neuronal activity in the hippocampus; this suggests using hippocampal slices as a model for studies of neurophysiological mechanisms of hereditary predisposition to catalepsy.

Key Words: *catalepsy; hippocampus; epileptiform activity*

Catalepsy is a state of long-term immobility with plastic muscle tone; it is an element of defense behavior and is associated with fear [6]. The extreme form of catalepsy is a syndrome of severe nervous dysfunction [11]. Immobility induced by dopamine D_2 receptor antagonists is a widely used model of catalepsy [9]. In 50% CBA mice, pronounced freezing reaction can be induced by repeated pinching of the skin of the nape of the neck (pinch-induced catalepsy), due to which these animals are widely used as a model for studies of molecular mechanisms of natural catalepsy [2,7]. A promising approach to detection of the relationship between catalepsy markers and nervous system function is the search for electrophysiological correlates in catalepsy. Significant intrastrain (paratypical) variability by the intensity of freezing reaction suggests that the studies can be carried out within the same animal strain, due to which the relationships between the detected correlates and the studied phenomenon can be established with higher reliability.

We studied the probability of epileptiform discharges reflecting synchronous discharge activity of pyramidal neurons [5,14] in the hippocampal slices of CBA mice with different predisposition to catalepsy.

MATERIALS AND METHODS

Electrophysiological experiments were carried out on hippocampal slices of 3-7-month-old male CBA mice, divided into 2 groups: 11 animals with high predisposition to catalepsy (HC) and 12 with low predisposition (LC). The selection procedure consisted in 10 test pinches of the neck [8]. The animals responding to the stimulus by at least 20 sec long cataleptic reaction in three tests were included into the HC group, other animals were referred to the group with low predisposition to catalepsy (LC group). The relationship between the duration of freezing and number of stimuli during testing was evaluated on random samples of 44 HC and 49 LC animals.

The experiments were carried out no sooner than 2 days after testing. After decapitation the

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brain was rapidly removed and plunged in cold carbogen-aerated (95% O_2, 5% CO_2) saline containing (mM): 125 NaCl, 3.8 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. The slices (350 μ) were placed into a flow-type (2 ml/min) experimental cell (11 ml) at 20-23°C. The solution in the cell was aerated and mixed with carbogen by means of aerolift. The temperature in the cell was heated to 32.0±0.5°C over 1 h. Extracellular recording electrode filled with saline was placed in the CA1 pyramidal layer. The recording of spontaneous electric activity of the hippocampus was started 120 min after preparation of the slices. After 30-min recording of basal activity, the perfusion solution was replaced with a solution without $MgSO_4$. The time of the experiment was divided into 5-min intervals and the mean frequency of the appearance of epileptiform discharges was calculated for each interval.

The results were presented as the mean and standard error of the mean ($M\pm m$). The significance of differences between the groups was evaluated by the analysis of dispersions (ANOVA) and Student's t test.

RESULTS

Spontaneous epileptiform discharges in our saline sometimes developed even without Mg^{2+}. Rare discharges (0.3±0.2 min^{-1}, n=11) were observed in the hippocampal slices of HC animals during 30-min recording of basal activity, but not in slices from LC animals. The difference between the groups was negligible under these conditions (p>0.14).

However, the reaction of slices to a decrease in Mg^{2+} concentration in the medium differed significantly in HC and LC mice (Fig. 1). Reduction of Mg^{2+} concentration in the solution increases cell sensitivity and frequency of epileptiform activity, including that in the hippocampus [10]. Gradual removal of Mg^{2+} from the medium was paralleled by a smooth increase in the incidence of spontaneous epileptiform activity and appearance of activity in previously silent slices, this process being significantly delayed in LC animals in comparison with HC ones. Two-way ANOVA with repeated measurements in factor 2 (time) detected a significant interaction between factors ($F_{3.66}$=5.86; p<0.002) during min 0-20 of perfusion with magnesium-free solution, which confirmed the significance of the detected differences in the time course of epileptiform activity formation in HC and LC mice with reduction of Mg^{2+} concentration in the medium. More rapid increase in activity in slices from HC animals led to a significantly higher incidence of

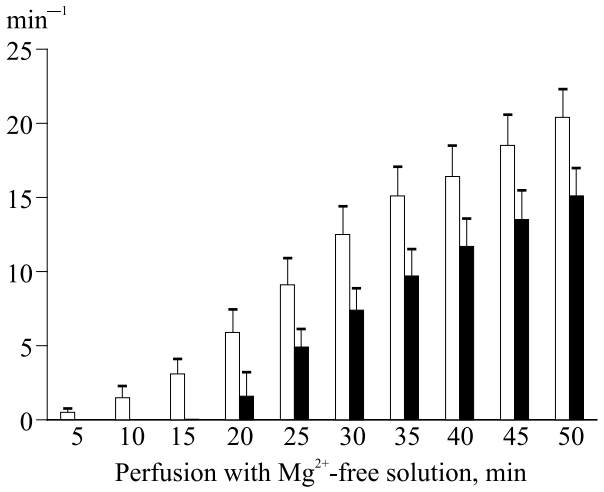

Fig. 1. Time course of increase in the epileptiform activity (mean frequency of epileptiform discharges) in hippocampal slices of HC and LC mice during reduction of Mg^{2+} concentration in the medium. Light bars: HC animals; dark bars: LC animals.

population discharges in this group in comparison with LC animals (the group factor effect for this period was also significant: $F_{1.22}$=9.50; p<0.006).

During the next period (min 15-50 of perfusion with magnesium-free solution), the rate of discharges was virtually the same in the two groups (interfactorial interaction was negligible). The frequency of discharges in HC mice was retained at a higher level than in LC ones ($F_{1,22}$=4.51; p<0.046).

The hippocampus is not a structure directly involved in motor regulation and development of catalepsy [1,4]. Presumably, the factors determining high predisposition to catalepsy in CBA mice modulate the work of many brain compartments irrespective of their specialization, which can be the main cause of the detected correlation between predisposition to catalepsy and hippocampal activity.

On the other hand, the possibility of indirect involvement of the hippocampus closely connected with the training mechanisms [12] in catalepsy mechanism cannot be ruled out, which is confirmed by the practice of using conditioned reflexes for the creation of experimental catalepsy models [3]. During testing of predisposition to catalepsy in CBA mice, the duration of immobilization increased from one stimulus to another in both HC and LC mice (Fig. 2; Pearson's coefficients of correlations r=0.47; p<0.01; n=44 and r=0.2; p<0.001; n=49, respectively). It is remarkable that animals of both groups extremely rarely demonstrated freezing reaction in response to the first stimulus and the difference between the groups manifested as a result of more rapid progress of the reaction duration in HC animals (bifactorial ANOVA (group(2)×time(10)) with repeated measurements in factor 2 showed a signi-

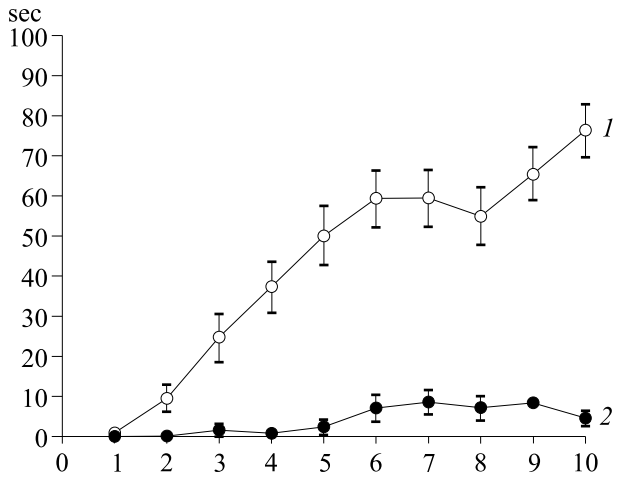

Fig. 2. Increased length of cataleptic reaction during testing in CBA mice. *1*) HC mice, *2*) LC mice. Abscissa: test stimulus No.; ordinate: duration of freezing reaction (period of observation did not exceed 120 sec).

ficant interaction between the factors: $F_{9.819}=17.7$, $p<<0.001$). Hence, the involvement of training processes in the formation of pinch catalepsy in mice is highly probable.

The development of the epileptiform activity phenomenon in hippocampal slices is associated with synchronization of discharge activity of the pyramidal neurons [5]. Speaking about possible contribution of training to the formation of pinch catalepsy, we should like to note the presumable role of synchronization of hippocampal neuronal activity in the formation of memory traces [13] and the correlation between mouse training capacity and probability of population discharges in the hippocampal slices [14]. A possible mechanism of catalepsy predisposition is (along with reduced freezing reaction threshold [1]) changed excitability of neurons and/or network organization of the hippocampus, resulting in facilitation of neuronal activity synchronization and hence, in facilitation of conditioned reflex relationships formation.

Hence, our data indicate that some factors determining the predisposition to catalepsy are essential for the neuronal activity synchronization in the hippocampus, which suggests the hippocampal slices as a prospective model for studies of the mechanisms of neuronal activity modulation by factors responsible for the formation of predisposition to pinch catalepsy in mice.

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