PHYSIOLOGY

Peculiarities of Latent Inhibition Formation in SHR Rats in Conditioned Task of Different Complexity

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Inhibition of attention to irrelevant stimuli was studied in SHR rats using latent inhibition test. Latent inhibition was formed in two types of conditioned tasks with different levels of complexity and stress. Passive and active avoidance conditioning was preceded by preexposure to conditioned stimulus consisting of 20 and 100 non-reinforced presentations, respectively. Control Wistar rats demonstrated successful formation of latent inhibition in both tasks. SHR rats showed different degree of disruption of latent inhibition depending on the type of behavioral task. We assume that learning defect in these animals in respect to both novel and preexposed conditioned stimuli is associated with the lack of behavioral inhibition.

Key Words: *latent inhibition; passive and active avoidance; SHR rats*

Spontaneously hypertensive rats (SHR rats) are used as the model of human hypertension and cardiovascular diseases. Other unique phenotypic characteristics of these animals: hyperactivity, impulsivity, and attention deficiency, are similar to the main triad of behavioral symptoms of pediatric neuropsychiatric disorder (attention deficit hyperactivity disorder) [10]. In numerous studies SHR rats are used as a genetic model of attention deficit hyperactivity syndrome for elucidation of its neurobiological and other mechanisms. Disturbances in conditioned activity in these animals are unequivocal in any behavioral tasks from simple recognition (social memory) to complex discrimination in mazes [1,2,4,8,9]. Apart from poor learning, prolonged retention of memory trace was observed in repeated conditioned passive avoidance testing in SHR rats [1], which could be stipulated by inadequate maintenance of attention to non-reinforced conditioned stimulus. Maintenance of attention to irrelevant elements of the tasks in similar situation was also observed in children with attention deficit syndrome [11]. Advantages and drawbacks of attention *vs*. inattention appear to be biologically adaptive processes, since they provide the background for activation, maintenance, and switching of this important function [7]. To test the ability for attention switch, the phenomenon of latent inhibition (LI) is used, which is characterized by the delay of condition reaction formation in healthy subjects as a result of multiple preexposure (PE) of conditioned stimulus before training [5]. LI is known to be affected in children with attention deficit hyperactivity syndrome [6]. The same result was obtained in attempt to form LI in SHR rats [3]. Authors used the model of contextual fear conditioning, when the possibility to avoid punishment was excluded. In view of lack of other studies focused on this issue, as well as due to dependence of LI development on the type of training, we compared the formation LI in SHR in conditioned passive and active avoidance tasks.

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MATERIALS AND METHODS

The experiments were performed on adult male rats (250-360 g) of two strains, hypertensive SHR and normotensive Wistar rats, obtained from the Laboratory of Animal Breeding, Institute of Cytology and Genetics, Siberian Division, Russian Academy of Sciences. The animals were kept in plastic cages (2 animals per 1 cage) under standard day-night cycle and with free access to water and food. The adaptation period before the experiment was 10 days. The experiments were carried out with strict adherence to humanity principles in accordance to "Rules for studies with experimental animals" (Appendix to Order of Ministry of Health USSR No. 755, 12.08.1977), approved by Institutional Review Board, Institute of Physiology, Siberian Division, Russian Academy of Medical Sciences.

Passive and active avoidance conditioning was carried out in two situations: to a novel conditioned stimulus and after extinction of the novelty at the stage of PE before training (conditioning). Before each experiment, the animals were divided into groups (n=9-14) with PE or without PE.

In case of passive avoidance conditioning, PE was carried out for 5 days with placement of the rat into the experimental chamber 4 times per day (contextual conditioned stimulus). On day 6 (stage of conditioning), the transfer into the dark chamber was associated with electric shock (0.75 mA, 2 sec) and the animal was immediately returned to home cage. Conditioned reaction was tested in 24 h with registration the latent period (LP) for the transfer into the unsafe compartment. Animal behavior was monitored for 180 sec in each session.

Active avoidance conditioning was carried out in other animals in automated shuttle box equipped with IFT-04 software. Before training, the control animals (without PE) adapted to the training box for 3 min and then electric bulb (10 W) was turned on in the compartment where the rat was and 5 sec later electric current (0.7 mA) was delivered to the grid floor in this compartment; the current was turned off simultaneously with the light after rat transfer into the other compartment. Time interval between presentations of the conditioned and unconditioned stimuli varied from 22 to 30 sec. Training was carried out in one session consisting in 100 presentations of light+shock. The following parameters were automatically recorded: LP of transfer in response to pain stimulus (escape reaction), the number of light+shock presentations before first conditioned escape reaction (parameter 1), and the number of stimulus pairings before consolidation of conditioned avoidance reaction (7 consecutive right answers, parameter 2). To obtain the LI effect, PE to conditioned light stimulus (100 PE) was carried out 1 day before the training. It was similar to that in the training day, but without unconditioned stimulus. The presence of LI was determined by comparing groups with and without PE.

The data were analyzed using two-way ANOVA (ANOVA 6.0). Rat strain and PE were used as independent factors. Effect of each factor was assessed using Fisher's test and significance of between-group differences of mean values was determined using LSD method.

RESULTS

Results of passive avoidance conditioning in rats after 20 PE or without PE in terms of two-way analysis of variations of LP for passing into the unsafe compartment 1 day after the training (Fig. 1) demonstrated significance of the strain factor ($F_{1.41}$=15.25, p<0.000) and PE factor ($F_{1.41}$=14.56, p<0.000), but not of their interaction ($F_{1.41}$=2.30, p=0.13). Post-hoc analysis revealed significant shortening of the time of transfer in Wistar rats after PE (p=0.001) in comparison with rats without PE, what reflects LI effect. In SHR, the intragroup differences between animals after 20 PE and without PE were insignificant ($F_{1.41}$=3.25, p=0.06), but close to statistical significance. This fact may be indicative for the trend toward LI development or for the possibility of its development in behavioral task. Interstrain comparisons revealed highly significant differences in LP of transfer only for Wistar rats and SHR without PE ($F_{1.41}$=15.54, p=0.000), which supports the data on disturbances in passive avoidance conditioning in SHR [1].

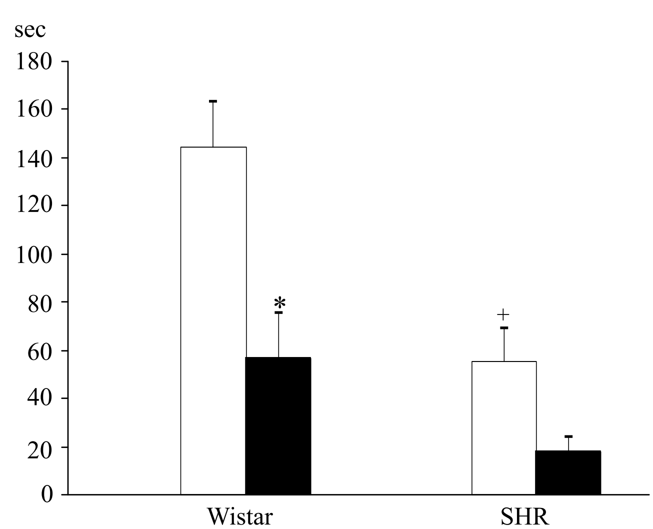

Fig. 1. Effect of conditioned stimulus PE on passive avoidance conditioning training. Ordinate: LP of transfer into unsafe compartment of the chamber. Light bars: training without PE; dark bars: training after 20 PE. *p<0.01 in comparison with the control group without PE, +p<0.01 in comparison with Wistar rats.

In the case of active avoidance conditioning, general analysis of the differences in the number of pairings for parameter 1 showed significance only for PE factor ($F_{1.34}$=8.95, p<0.001); in case of parameter 2, significance has been shown for PE ($F_{1.34}$=11.76, p=0.002) and PE—strain interaction ($F_{1.34}$=6.11, p=0.02). Significant effect of PE on training was detected only in Wistar rats for both parameter 1 ($F_{1.68}$=7.37, p=0.009) and parameter 2 ($F_{1.68}$=13.56, p=0.0005) in comparisons with the corresponding groups without PE (Fig. 2). The same comparisons for SHR revealed no significant differences, which confirm impaired LI. Disturbances in passive avoidance conditioning in SHR (without PE) can be estimated using parameter 2 ($F_{1.64}$=4.98, p=0.029 in comparison with group of Wistar rats without PE).

To observe the dynamics of the training process, the mean LP values for transfer in response to shock were found in first 5 sessions, 10 light+shock pairings per each session (Fig. 3). Two-way ANOVA for repeated measures revealed significance of group factor ($F_{3.35}$=4.94, p<0.01), session factor ($F_{4.140}$=38.00, p<0.000), and for their interaction ($F_{4.140}$=3.62, p<0.000), which was determined by different reaction to PE in two rat strains. Post-hoc comparisons revealed significant differences in transfer LP in response to shock in Wistar rats (group 1 and group 2) in sessions 1, 2, 3, and 5, *i.e.* the time of reaction to painful stimulation significantly increased after PE. Despite of observed delay of shock escape reaction in group 2, the mean transfer LP decreased; significant differences were found even between sessions and 2 (p<0.01) and these differences were preserved until the end of observation. In SHR (groups 3 and 4), no intergroup and intragroup differences were found, which attests to the absence of PE effect on punishment escape.

Although LI disturbances were registered in SHR in both behavioral tasks, their degree was different, which can be explained by not only learning difficulties, but also the differences in the procedures of non-reinforces conditioned stimulus. Previous analysis of different PE protocols showed that the number of placements into the chamber before the training, rather the time spent in the chamber, is important for the stability of LI effect. It is not inconceivable that the habituation to the new stimulus for its transfer in to the group of irrelevant stimuli requires more PE for SHR. Anyway, the trend toward LI development in CPA may be interpreted as the possibility of its development in this context. LI disturbance in active avoidance was total with no differences in all 3 parameters when compared with the corresponding control without PE. Difficulties in habituation or in inhibition of attention to non-

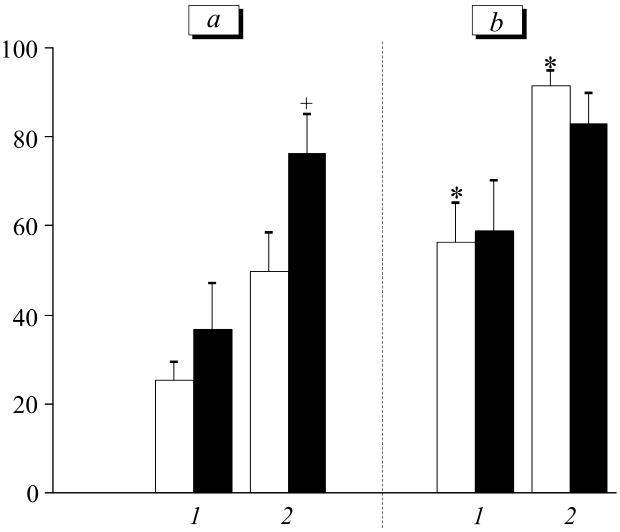

Fig. 2. Parameters of active avoidance training (without PE; *a*) and LI development (100 PE; *b*) in Wistar rats (light bars) and SHR (dark bars). *1*) number of light+shock pairings before the first correct response; *2*) the number of pairings before acquisition of conditioned reaction. *p<0.01 in comparison with the data obtained without PE; ^+p<0.01 in comparison with Wistar rats.

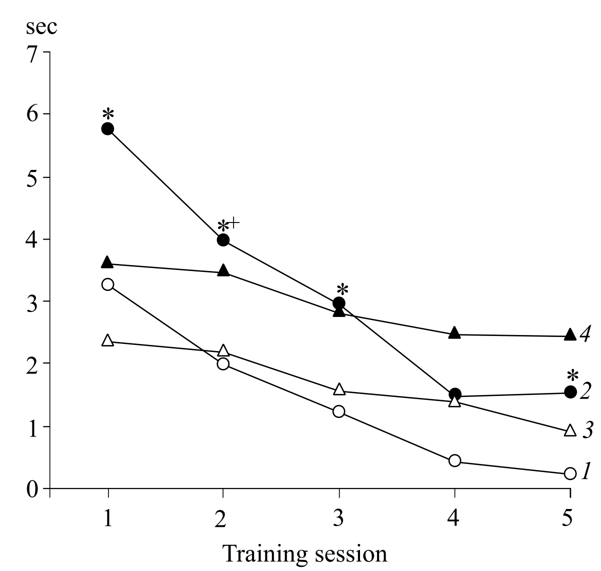

Fig. 3. Dynamics of transfer LP in response to painful stimulus in the first 5 sessions of active avoidance training. *1*) Wistar rats without PE; *2*) Wistar rats after 100 PE; *3*) SHR without PE; *4*) SHR after 100 PE. *p<0.01 in comparison with *1*; ^+p<0.05 in comparison with session 1 in Wistar rats *2*.

reinforced stimulus were also observed in estimating spontaneous passive avoidance extinction, acquired toward new stimulus [1]. In investigations of short-term social memory in SHR, the dishabituation in the time of exploration of unfamiliar individual was observed in both first and second presentation [9]. So, dishabituation appears to be the main obstacle for LI formation, as well as for learning at all. In our experiment it was evident in the delay of formation of punishment avoidance reaction (Fig. 3), the critical process in passive avoidance conditioning. Representations of LI as interferential inhibition or as switching attention from one situation "conditioned stimulus — no consequences" to another situation "conditioned stimulus — reinforcement" [12], are basically the same. In children with attention deficit disorder, the deficiency of interferential inhibition on the no-go event was observed with no differences in the answers on color stimuli in classic go/no-go task [11]. In this study we discussed motor inhibition deficit. Results of our work cannot be regarded solely from this point of view, since in one behavioral task the habit acquisition is provided by motor restrain, whereas another task requires motor activity. In addition, both processes — learning by novelty and LI — are disturbed in SHR. It appears that the approach we used may be helpful for further investigations of the relationship between LI in SHR and neurobiological and neurochemical substrates.

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