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Alterations in Conditioned Food-Procuring Activity Related to Long-Term Pentylenetetrazole Kindling in Rats

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We studied the mechanisms underlying impairment of conditioned food-procuring activity of rats in the state of long-term kindling induced by 120 injections of pentylenetetrazole (PTZ). In four animal groups, positive and negative modulation of the activity of the adrenergic and serotonergic neuromediatory systems was provided using introductions of ludiomil, α-methyl-para-tyrosine, L-tryptophan, or para-chlorophenylalanine. Realization of the conditioned food-procuring reflex was estimated in the test with a radial labyrinth on the 30th, 60th, and 90th days. In the dynamics of PTZ-induced kindling, we observed intensification of cognitive dysfunctions, which was manifested in worsening of the results of training for the conditioned reflex. Similar effects, but of a significantly lower intensity, were found in kindling rats with activation of the noradrenergic and serotonergic systems. Therefore, the state of the above-mentioned systems is important for the development of cognitive dysfunctions under conditions of long-term PTZ-induced kindling.

Keywords: kindling, pentylenetetrazole, conditioned reflex, cognitive dysfunctions, noradrenergic and serotonergic systems.

INTRODUCTION

Kindling-induced convulsions in animals are accompanied by the development of appreciable disorders of emotional behavior [1, 2], motor activity, and cognitive functions. During the interictal period, behavioral phenomena of stereotypic, defensive, and other types undergo noticeable modifications [3, 4]. The mechanisms underlying such disorders remain little studied.

The development of long-term pentylenetetrazole (PTZ)-induced kindling, except for clearly pronounced manifestations of convulsive activity [5], is accompanied by behavioral impairments with the predominance of a depressive component [5, 6]. In rats under conditions of long-term electrostimulation-induced kindling (90-100 electrical stimuli applied to the amygdala), researchers also observed modifications of emotional behavior with formation of the depressive state [7]. Based on these data, we aimed to elucidate peculiarities of food-procuring conditioned reflex activity (CRA) in the course of long-term PTZ-induced kindling, as well as neuropathophysiological mechanisms of such cognitive dysfunctions.

The monoaminergic theory is the leading concept related for the pathogenesis of depression, manifestations of which are the main phenomenon in long-term PTZ-induced kindling [8]. Considering this, we studied impairment of the food-procuring CRA in the model of PTZ-induced kindling under conditions of modulation of adrenergic and serotonergic neuromediator systems (NMSs).

METHODS

Studies were carried out on Wistar male rats in chronic experiments.

To activate and suppress the activity of the noradrenergic central system, we used, respectively, ludiomil (LD; Novartis Pharma AG, Switzerland) i.p. injected in a dose of 20 mg/kg for 14 days and α-methyl-para-tyrosine (AMPT; Sigma, USA) i.p. injected in a dose of 80 mg/kg for 3 days [9]. The activity of serotonergic NMS was activated or suppressed using, respectively, i.p. injections of L-tryptophan (L-T; Grodno Medical Preparations Plant, Belarus) in a dose of 100 mg/kg for 14 days [10] and para-chlorophenylalanine (PCPA; Sigma,

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USA) in a dose of 300 mg/kg for 3 days [11]. After modulation of the functional state of the adrenergic and serotonergic systems, we induced the state of PTZ kindling [5]. The number of PTZ injections (30-35 mg/kg) did not exceed 120.

The following 10 experimental groups were formed: 1, control (intact rats); 2, kindling rats; 3, LD + control; 4, LD+ kindling; 5, MPT + control; 6, MPT + kindling; 7, L-T + control; 8, L-T + kindling; 9, PCPA + control, and 10, PCPA + kindling. Each experimental group consisted of 10 rats; the control group included 8 rats.

The peculiarities of realization of the food-procuring conditioned reflex (CR) were studied in rats in the state of long-term kindling using an octactinal radial labyrinth (RL) according to the technique described earlier [12]. To create high food motivation, the studied rats were subjected to food deprivation; the body mass of these animals was reduced to 85% of the initial value using limitation of food but with free access to water. In the course of a training session, the rats learned, using external visual cues, to find food in a feedbox placed at the end of an arm. We recorded successions of visited arms of the labyrinth, number of arm entries up to the moment of entry of the rat into the "true" arm branch (with the feedbox containing food globules), and the duration of the presence of the rat in this branch. During one experimental day, the rat was allowed to realize 12 attempts to find food in the RL arm; then it was removed from the labyrinth. The experiment was discontinued in the case where the rat realized six successive successful attempts and found food or if the rat did not find food during 10 min. Experiments were carried out 24 h after injections of PTZ (in experimental groups) or saline (in the control groups).

The obtained data were treated statistically using one-variant ANOVA and (in a positive case) the Newman–Keuls *post-hoc* test. We considered $P < 0.05$ as a criterion for the significance of intergroup differences.

RESULTS AND DISCUSSION

Control rats found food in the RL at the first (or maximum, second) try. The formed CR was preserved during the entire duration of the experiment. Isolated injections of any drug modulating the activity of the noradrenergic or serotonergic mechanisms into control rats induced no significant modifications of their food-procuring activity. In kindling rats, 30 days after the beginning of PTZ injection, the mean number of attempts to find food was 10.9 and dramatically exceeded the analogous index in animals of the control group ($P < 0.001$). Within this time interval, the studied indices in rats with activation of the noradrenergic and serotonergic

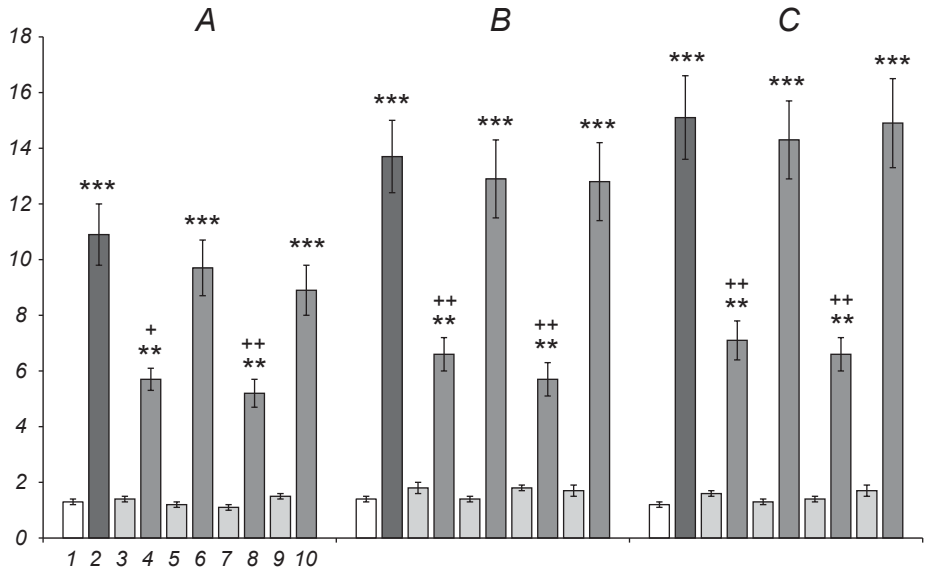

Fig. 1. Characteristics of conditioned reflex food-procuring activity according to the results of the test using a radial labyrinth in kindling rats under conditions of modulation of the activity of the noradrenergic and serotonergic central systems. Horizontal scale) Animal groups 1-10 according to those listed in the text (see Methods); vertical scale) mean number (± s.e.m.) of trials up to the moment of successful localization of food. Two and three asterisks indicate cases of significant differences of the studied indices with $*P < 0.01$ and $**P < 0.001$, respectively, as compared to those in the control group 1; one and two crosses indicate differences with $*P < 0.05$ and $**P < 0.01$ in comparison with group 2 (ANOVA + the Newman–Keuls test). Panels A, B, and C correspond to the data obtained on the 30th, 60th, and 90th days of the experiment, respectively.

central systems (groups 4 and 8, respectively) were 5.7 ± 0.4 and 5.2 ± 0.9; both these values were significantly greater ($P < 0.05$ and $P < 0.01$) than the analogous index in control rats (group 1) but much smaller than the respective index in kindling (group-2) animals ($P < 0.01$). The mean numbers of attempts to find food in the feedbox of the RL made by rats with pharmacological suppression of activity of the monoaminergic systems demonstrated no significant differences from the respective index in intact animals ($P > 0.05$) (Fig. 1A).

On the 60th experimental day, kindling rats of group 2 realized, on average, 13.7 ± 1.3 attempts for search for food, while 15.1 ± 1.5 attempts were made on the 90th day (i.e., these values were significantly greater than that in the control, $P < 0.001$). Under such conditions, the studied indices in rats with activation of the noradrenergic and serotonergic systems on the 60th day were 6.6 ± 0.6 and 5.3 ± 1.2, respectively, which were much greater than those in intact rats ($P < 0.01$) but, at the same time, significantly smaller ($P < 0.01$) than those in group 2 (Fig. 1B).

The nearly analogous results were observed on the 90th experimental day (Fig. 1C).

The obtained data are indicative of clearly pronounced impairments of the CRA in rats that were in the state of long-term PTZ-induced kindling. In the dynamics of this type of kindling, we observed certain progressive worsening of cognitive functions (Fig. 1A-C). The observed impairments of the CRA were also clearly recorded in the groups of rats with activation of the noradrenergic and serotonergic systems, but the intensity of the respective negative shifts was noticeably (and statistically significantly) lower. These facts agree, in general, with the well-known concept on the significant role of the monoaminergic NMSs in mechanisms underlying the development of memory and learning. It should be noted that, under conditions of our experiments, pharmacological suppression of the activity of the above systems did not result in significant modifications of food-procuring CRA; explanation of this fact needs further investigations.

Therefore, the development of long-term PTZ-induced kindling is related to the progressive impairment of the CRA, which is manifested in worsening of the formation and realization of food-procuring CR. The states of the noradrenergic and serotonergic central systems (the state of the latter system, to a somewhat greater extent) are of significant importance for the mechanisms underlying the development of cognitive impairments.

Experiments were carried out in accordance with the National and International Conventions for the Protection of Vertebrate Animals Used for Experiments, as well as with the regulations of the Bioethics Committee of the Odessa National Medical University (Ministry of Public Health of Ukraine).

The authors, O. O. Prishchepa,O. A, Shandra, and R. S. Vast'yanov, confirm that they have no conflict of interest.

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