Acquisition of an Active Avoidance Reaction in Rats and Morphological Changes in the Hippocampus in Pentylenetetrazol Kindling

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The relationship between measures of active avoidance behavior and morphological changes in the hippocampus was studied in rats after kindling induced by administration of pentylenetetrazol. Pentylenetetrazol kindling impaired the acquisition of the avoidance reaction and increased the number of intersignal reactions without altering the acquisition of the avoidance reaction in the shuttle box in rats. The numbers of neurons in the hippocampus (fields CA1 and CA3) and dentate fascia decreased, while the numbers of damaged neurons increased. Inverse correlations between seizure severity and the numbers of neurons in hippocampal fields CA1 and CA3 and the dentate fascia were seen in rats subjected to kindling. Rats of this group also showed positive correlations between seizure severity and the numbers of damaged neurons in field CA1 and the dentate fascia. There were no correlations between measures of convulsive activity or the number of cells in hippocampal zones and measures of the acquired avoidance behavior. Control animals showed negative correlations between the numbers of damaged cells in field CA1 and the dentate fascia and the characteristics of avoidance behavior in the first and third training sessions.

KEY WORDS: pentylenetetrazol kindling, neurodegeneration, hippocampus, active avoidance.

Kindling is a well known phenomenon whereby repeated electrical, chemical, or audiogenic stimuli of subconvulsive strength lead to the development of increased convulsive readiness [15, 20]. Repeated i.p. doses of pentylenetetrazol (PTZ) at a subthreshold dose lead to increases in convulsive activity. This is manifested externally as a change in convulsive reactions from mild twitching of the ears and whiskers to clonic-tonic seizures.

Short periods of convulsive activity evoked by electrical stimulation of the perforant path, olfactory bulbs, or amygdala were accompanied by loss of neurons in the dentate fascia (DF) and hippocampus (fields CA1 and CA3) [10, 11]. Similar results have been obtained with PTZ kindling [3, 4, 13, 21, 24, 25]. These data provide a significant level of reproduction of results obtained from studies using segments of brain tissue from humans suffering from temporal epilepsy [5, 8].

In animals, kindling, particularly that evoked by PTZ, is accompanied by changes in emotionality [13, 21] and cognitive functions [21]. Krivanek [19] was one of the first to demonstrate impairments or facilitation of the formation of avoidance reactions in mice after single administration of different PTZ doses. PTZ kindling usually leads to impairments in the acquisition of conditioned active avoidance reactions (CAAR) in rats [6, 14, 26]. Some authors believe that kindling-evoked cognitive deficit is due to the death of cells in the hippocampus [7, 25]. At the same time, it is well known that hippocampal lesions significantly facilitate acquisition of CAAR [1, 17, 21].

The aim of the present work was to study the relationship between measures of learning in rats in the CAAR test...
and morphological changes in the hippocampus of rats after PTZ kindling.

METHODS

Studies were performed using male Wistar rats (from Stolbovaya, Moscow Region) aged eight weeks and weighing 180–200 g at the beginning of the experiment. Rats were kept in conditions of an artificial light regime (day from 08:00 to 20:00) in groups of six individuals per cage with free access to water and food. Experiments were performed in accordance with international regulations for studies involving laboratory animals using a protocol approved by the Ethics Commission of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences. Animals were randomized to two groups of 12 individuals. One group was subjected to the kindling procedure using i.p. doses of pentylenetetrazol (Sigma, USA). PTZ dissolved in isotonic NaCl was given to rats at a dose of 40 mg/kg with intervals of 48 h (with breaks on rest days) for four weeks (a total of 13 injections). Control animals received an equivalent volume of solvent. The convulsive signs of seizures were assessed during the 20 min following doses using a scale described previously [13].

Seven days after completion of kindling, rats were trained to a CAAR in a shuttle box. The conditioned stimulus (CS) was a flash of light (6-W bulb) and the unconditioned stimulus (UCS) consisted of electrocutaneous stimulation of the paw (trains of impulses of duration 400 msec, 50 Hz, 1.5 mA). Training was performed in a Multiscreen-4 apparatus (USSR) connected to a personal computer. Animals were adapted to the chamber for 5 min before training started. Each session started with presentation of the CS. The UCS was presented 5 sec after the onset of the CS. Both stimuli acted simultaneously for 40 sec or until the rat transferred from this sector to the other, safe sector. Interstimulus intervals were 30 sec. The procedure was repeated at the end of this time. Rats were presented with 30 combinations of the CS and UCS each day. The CAAR training procedure was performed for three days.

Once training was complete, the rats were anesthetized with chloral hydrate and brains were fixed by perfusion with formalin:acetic acid:ethanol (2:1:7) solution. Brains were extracted, further fixed for 2 h, and stored in 70% ethanol. Blocks containing the hippocampus were embedded in Paraplast. Frontal sections of thickness 10 μm, stained with cresyl violet, were studied. Quantitative counts were performed using four sections (from –3 to –4 mm from the bregma) located 250 μm from each other. The first section was selected by objective random selection. The counting method has been described in detail elsewhere [28]. The effects of stress associated with CAAR acquisition were assessed using animals subjected to kindling (n = 6) and an active control group (n = 12) consisting of rats presented with the same number of non-combined CS and UCS as the experimental rats.

Results were analyzed statistically using two-factor analysis of variance (with “group” and “session” as the factors). Histological data were analyzed using the Student and Mann–Whitney tests, depending on the distribution of the set. Relationships between measures were evaluated by calculating Spearman correlation coefficients.

RESULTS

Chronic PTZ at subconvulsant doses led to increases in the convulsive readiness of the rats. The development of kindling was apparent as a gradual increase in convulsions from minor ear and snout twitches to severe clonic seizures. Convulsive activity reached a plateau after the seventh PTZ injection, subsequently remaining essentially unchanged (the median convulsion intensity was 3 points on the scale described in [13]). Persistence of elevated convulsive readiness was tested ten days after completion of kindling by giving the animals PTZ at a subconvulsive dose. The rats demonstrated convulsions which were no different in terms of intensity from those occurring after the last kindling injection. Four rats demonstrated stage 4 (clonic-tonic seizures), while the remainder showed stage 3 (clonic seizures).

A CAAR is a complex form of behavior, which includes several different types of response. The following measures were used to assess behavior from all the possible reaction types: 1) transfer to the safe sector of the box after presentation of the UCS (escape); 2) transfer to the safe section of the box after presentation of the CS and before activation of the UCS (avoidance); 3) intersignal reactions (transfer from sector to sector during the intersignal interval). The appearance of escape reactions usually preceded the formation of avoidance reactions. Escape from the UCS could be apparent as directed running by the rat into the other sector after application of the UCS or as jumping and moving in a disordered fashion around the sector during exposure to the UCS. This latter type of escape reaction reflects the animals’ response to the use of a painful stimulus and was not dependent on the experimental conditions. This type of reaction to the UCS was not significantly different in animals the groups studied here, indicating that kindling had no effect on pain sensitivity.

Acquisition of the directed escape reaction showed no differences between the control and experimental groups during the initial period of training (Fig. 1, A). The number of escapes in the control group increased gradually, showing that these rats acquired this type of conditioned reflex reaction. In rats subjected to kindling, the number of escape reactions was significantly smaller than that in control animals (the effect of the “group” factor, F1,20 = 5.3, p < 0.03). The number of escape reactions was independent of the