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EFFECT OF PRENATAL PENTYLENETETRAZOL-INDUCED KINDLING ON LEARNING AND MEMORY OF MALE OFFSPRING

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Abstract—Current data concerning the effects of maternal seizure during pregnancy on newborns are limited. This study was carried out to investigate the effect of prenatal pentylenetetrazol (PTZ)-induced kindling on learning and memory of offspring. Female Wistar rats were kindled with i.p. injections of 25 mg/kg of PTZ on day 13 of their pregnancy. The spatial performance and passive avoidance learning of pups were tested at 7 weeks and 12 weeks of age using Morris water maze (MWM) task and shuttle-box apparatus, respectively. We found, for the first time, that prenatal exposure to maternal seizure induced by PTZ leads to a significant impairment of learning and memory. In addition, the number of live birth was significantly lower in kindled rats compared to control. In MWM studies, the young offspring of kindled rats had poor spatial learning ability. The frequent tonic-clonic seizures in pregnancy was also associated with a poor memory as evidenced by decrease in distance swam in the target quadrant by the offspring of the kindled mother in the adulthood. Data obtained from shuttle-box studies showed that retention latencies of pups born to kindled dams were significantly reduced compared to those born to control dams. The hippocampus, amygdala and frontal cortex are very important for memory consolidation and our data suggest that subsequent developmental events are not sufficient to overcome the adverse effects of prenatal exposure to maternal seizures to these regions of the brain. These observations may have clinical implications for cognitive and memory dysfunction associated with epilepsy during pregnancy. Published by Elsevier Ltd on behalf of IBRO.

Key words: prenatal seizure, pentylenetetrazol, Morris water maze, shuttle-box, learning and memory.

Epilepsy is one of the most common chronic diseases affecting over 1 million woman of child bearing age in the USA (Anderson et al., 1999; Bittigau et al., 2003). The infants of epileptic mothers are in higher risk for a variety of adverse pregnancy outcomes. Generalized tonic-clonic seizures increase the risk for hypoxia and acidosis, which can lead to irreversible damage to the CNS as well as to the other organs (Stumpf and Frost, 1978; Hallak et al., 1999; Lajoie and Mosche, 2004). Most children born to women with epilepsy are normal, but there is an increased risk of abnormal functional neurodevelopment in these children. Two to six percent of children born to an epileptic mother have a developmental delay (Koch et al., 1999) and have 2–7 fold higher rates of mental retardation than controls (Speidel and Meadow, 1972). Recent studies also indicate that generalized tonic–clonic seizures might result in cognitive problems for the child later in life, a phenomenon that is supported by evidence of significantly decreased verbal IQ scores in children whose mothers had more frequent generalized tonic–clonic seizures during pregnancy (Adab et al., 2004). Although, it has been suggested that maternal epilepsy may alter the morphology of the hippocampus of newborns, leading to cognitive impairment (Baka et al., 2004), the effects of epileptic activity on different areas on the brain have not been clearly established (Sederberg-Olsen and Olsen, 1983; Schneiderman et al., 1994). Clinical studies in this area are often difficult to interpret and compare due to exposure of fetus to antiepileptic drugs, possible influence of socio-economical factors, and parental educational level on the outcome of exposed children. Experimental animal studies can be useful in investigating the effects of seizures on neurodevelopment and in identifying the potential mechanisms involved.

A periodic systemic injection of a convulsant drug, such as pentylenetetrazol (PTZ) (Assouline et al., 1984) has been shown to induce seizures in animal (Kilbey et al., 1979). PTZ is a noncompetitive antagonist of $GABA_A$ receptors. PTZ induced kindling is an accepted animal model for the study of epilepsy and its consequences on memory (Chen et al., 2003). Lamberty and Klitgaard (2000) have reported that PTZ-induced kindling disrupts spatial memory. It has been shown that kindling induced seizures cause hippocampal atrophy and neuronal loss in the limbic area (Cavazos and Sutula, 1990; Pitkanen et al., 1998), leading to learning deficits and cognitive impairment in the animal (Hamm et al., 1995; Gilbert et al., 2000; Mortazavi et al., 2005; Omrani et al., 2007), the effect of maternal seizure on the cognitive performance of newborns is, however, unknown.

In this report we examined the effects of prenatal exposure to the maternal tonic-clonic seizure on the learning and memory of adolescents and adult male rats. We found, for the first time, that offspring born to epileptic mothers have significantly impaired spatial memory and passive avoidance learning compared to those born to healthy mother. These findings, thus, provide a mechanistic link

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Abbreviations: ANOVA, analysis of variance; MWM, Morris water maze; PTZ, pentylenetetrazol.

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between maternal epilepsy and prenatal brain development and reveal a new aspect of PTZ modulation on learning and memory.

EXPERIMENTAL PROCEDURES

In vivo experiments

All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of the Laboratory Animals and approved by a local Animal Ethics Committee. All efforts were made to minimize the number of animals used and their suffering. Male and female Wistar rats (Razi Institute, Tehran, Islamic Republic of Iran) aged 3–4 months and weighting 250–300 g at the beginning of the experiments were used. The rats were kept in animal facility room (20–22 °C) on the 12-h light/dark cycle (lights on at 07:00 AM). Animals were housed singly in 24×24×45 cm^3 transparent, plastic cage, with food and water provided *ad libitum*. Female rats were randomly paired with males one day after delivery to the animal care facility. Vaginal smears were performed on the females every morning (07:00–08:00 AM) to look for the presence of sperm. The presence of sperm marked day 1 of pregnancy (Edwards et al., 2002). Each pregnant rat was transported to her home cage and stored singly.

Kindling

To test the impact of prenatal seizure on memory, on embryonic day 13 (E13) (E0 being the day in which positive vaginal smear was observed) (Baka et al., 2004), the pregnant dams were divided into two groups consisting of 10 animals each. Animals in the first group were kindled by i.p. injections of 25 mg/kg PTZ (sigma, St. Louis, MO, USA), 1 ml/kg body-weight every 15 min until seizures occurred. The total dosage of PTZ, however, did not exceed 75 mg/kg (three injections) (Kliouevaet al., 2001). Pilot study showed that this dose of PTZ injected every 15 min is optimal for kindling. Immediately after injection, seizure activity was observed for 45 min and scored according to Racine (1972), modified by Becker et al. (1992), as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic jerks without rearing; stage 3, myoclonic jerks, rearing; stage 4, turn over into side position, clonic-tonic seizures; stage 5, turn over into back position, generalized clonic-tonic seizures. Animals were considered to be kindled after reaching stage 4 or 5 seizures. The animals in the second group (the control group) received an equivalent volume of normal saline on a similar schedule. After parturition, pups were counted for each litter and weighted. Pups were then housed with their littermates until weaning at postnatal day 21 (Huang et al., 2002). Male pups from each litter were stored singly in a cage. To reduce possible litter effects, a total of four male pups from any litter were used in this experiment. Animals in each group, that is, kindled (K) and the control (C) groups were then divided into two subgroups of 16 males each according to Ehman et al. (2007). The first subgroups were allowed to grow to ~7 weeks (K7 and C7; adolescents) and the second subgroups were allowed to grow to 12 weeks of age (K12 and C12; adult). Within each of these subgroups, half of the rats were used for testing in the Morris water maze (MWM), while the other half was tested in the shuttle-box apparatus.

Behavioral assessment

Morris water maze (MWM) apparatus. The MWM consisted of a dark circular pool of 140 cm in diameter and 70 cm in height. The pool was filled to a height of 35 cm of water, 22±2 °C. A transparent Plexiglas escape platform (10 cm in diameter) was located below the water surface. Pilot experiment showed that platform was not invisible to the rats. The apparatus was located in a room with numerous extramaze cues that remained constant throughout the experiment. The distance swum to the platform (swim length), and the distance spent in each quadrant was recorded by a video tracking system.

Procedure. The MWM procedure was done according to Omrani et al. (2007). Briefly, on the first day, rats were placed on the escape platform for 60 s, which was at the center of the empty pool and on the second day the rats were placed again on the platform in the same position with the pool being full of water. When the rat climbed off the platform, it was guided back on to the platform. Training started the following day. During this period, the escape platform was located in the center of the northwest quadrant and all rats were given a daily session of four trials for six consecutive days. On each trial the rat was placed in the water facing the pool wall at one of four randomly determined starting locations (north, west, east or south poles). Once the rat located the platform, it was allowed to stay on it for 30 s. If the rat was not able to find the platform within 60 s, it was guided to it and allowed to remain on it for 30 s. The rat was then returned to its heated cage for a 30 s inter-trial interval. Twenty-four hours after last training trial, spatial memory was examined in a probe trial. During this trial, the platform was removed from the pool and the rats were allowed to swim freely for 60 s. The distance spent swimming in the quadrant where the platform was previously located was recorded. To assess whether any motivational factors interfered with the rat's ability to escape, 24 h after probe test, a visible platform trial was designed in which escape could be guided by proximal rather than distal spatial cues (Omrani et al., 2007). During this trial, the platform was raised above the water surface and placed in the southeast quadrant and extra maze cues were removed from the walls and the rats were allowed to swim freely for 60 s. The distance to platform (swim length) was recorded (Gilbert et al., 2000).

Passive avoidance apparatus. The passive avoidance apparatus consisted of two light (Plexiglas) and dark (Black) compartments of the same size (20×20×30 cm^3) separated by a door. The floor of the dark compartment was made of stainless-steel bars (0.5 cm diameter) separated by a distance of 1 cm. Intermittent electric shocks (50 Hz, 3 s), 1 mA intensity, were delivered to the floor of the dark compartment by an isolated stimulator (Jafari-Sabet, 2006).

Inhibitory-avoidance training. The rats were allowed to become familiar with the laboratory environment 1 h before each of the training or testing sessions. All training and testing were carried out between 08:00 AM and 12:00 AM. Each animal was placed in the light compartment for 20 s, after which the door was raised and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. The animal was removed from the experiment when it waited for more than 180 s to cross to the other side. Once the animal completely crossed to the next compartment, the door was closed and a 1 mA foot shock was delivered for 3 s. The rat was then removed from the apparatus and 2 min later, the procedure was repeated. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. All the animals were trained with a maximum of two trials (Jafari-Sabet, 2006).

Retention test. Twenty-four hours after training, a retention test was performed to examine long-term memory. Each animal was placed in the light compartment for 20 s, the door was opened, and the latency for entering into the shock compartment (as described in the training session) was measured as step through latency (STL). During these sessions, no electric shock was applied and the test session ended when the animal entered the shock compartment or remained in the light compartment for 600 s (criterion for retention) (Jafari-Sabet, 2006).