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EFFECT OF CENTRAL MUSCARINIC RECEPTORS ON PASSIVE-AVOIDANCE LEARNING DEFICITS INDUCED BY PRENATAL PENTYLENETETRAZOL KINDLING IN MALE OFFSPRING

A. POURMOTABBED, [a,b] **G. MAHMOODI,** [c*] **S. MAHMOODI,** [d] **A. MOHAMMADI-FARANI,** [d,e] **S. E. NEDAEI,** [f] **T. POURMOTABBED** [g] **AND T. POURMOTABBED** [h]

[a] *School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

[b] *Applied Neuroscience Research Center, Bagiyatallah (a.s.) University of Medical Sciences, Tehran, Iran*

[c] *Department of Experimental Science, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran*

[d] *Department of Pharmacology and Toxicology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran*

[e] *Novel Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*

[f] *Department of Neuroscience, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran*

[g] *Kermanshah University of Technology, Kermanshah, Iran*

[h] *Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA*

Abstract—Occurrence of the epileptic seizures during gestation might affect the neurodevelopment of the fetus resulting in cognitive problems for the child later in life. We have previously reported that prenatal pentylenetetrazol (PTZ)-kindling induces learning and memory deficits in the children born to kindled mothers, later in life but the mechanisms involved in this processes are unknown. The cholinergic system plays a major role in learning and memory. The present study was performed to investigate the possible involvement of central muscarinic cholinergic receptors on learning and memory deficits induced by prenatal PTZ-kindling in male offspring. Pregnant Wistar rats were kindled by repetitive i.p. injection of 25 mg/kg of PTZ on day 13 of their pregnancy. The effect of intracerebroventricular (ICV) microinjection of scopolamine and pilocarpine, muscarinic cholinergic receptors antagonist and agonist, respectively on passive-avoidance learning of pups were tested at 12 weeks of age using shuttle-box apparatus. Our data showed that the retention latencies of pups that received scopolamine (2 or 3 μg) were significantly reduced compared to those received normal saline ($p < 0.05$). Interestingly, post training ICV administration of pilocarpine (2 μg) retrieved pups' memory deficits ($p < 0.001$). These results demonstrate for the first time, the importance of the central muscarinic cholinergic receptors in learning and memory deficits in pups born to kindled dams and suggest a central mechanism for the cognitive and memory dysfunction, associated with seizures during pregnancy.

Key words: prenatal seizure, passive avoidance learning, scopolamine, pilocarpine, rat.

INTRODUCTION

Epilepsy is one of the neurological disorders in pregnant women (Hvas et al., 2000) which results in an increased risk of preterm delivery, affects the development of the fetus (Koch et al., 1999; LaJoie and Moshe, 2004; Chen et al., 2009; Mawer et al., 2010) leading to offspring with low birth weight (Speidel and Meadow, 1972; Hvas et al., 2000; Brewer and Waltman, 2003) and decreased verbal IQ scores later in life (Adab et al., 2004). To the best of our knowledge, the effect of maternal seizures on neurotransmitter systems in the developing brain of the fetus and its consequence on cognitive functions of the child is poorly understood. We have previously reported that exposure to maternal seizures during gestation clearly impairs spatial and also passive avoidance learning in the adulthood (Pourmotabbed et al., 2011). However, the neural mechanisms involved have not been identified.

The muscarinic cholinergic receptors (mAChRs) are G protein-coupled receptors that are expressed in the brain and a variety of epithelial cells (Caulfield, 1993) and play important roles in central functions such as learning and memory (Gibbs, 1999), cardiovascular and respiratory regulation (Kinney et al., 1995). The role of the cholinergic system in learning and memory is well documented. (Bammer, 1982; Everitt and Robbins, 1997; Sarter and Bruno, 1997; Ye et al., 2001). M3-muscarinic receptor knockout mice show a deficit in fear conditioning learning and memory (Poulin et al., 2010) Miranda and co-worker have shown that cholinergic activity in the insular cortex is necessary for acquisition and consolidation of contextual memory (Miranda and Bermudez-Rattoni, 2007). In addition, it has been reported that pre- and post-training systemic administration of muscarinic agonist enhance performance of inhibitory avoid (Baratti et al., 1979),

*Corresponding author. Tel: +98-831-4274618; fax: +98-831-4274623.
E-mail address: g.mahmoudi@yahoo.com (G. Mahmoodi).
Abbreviations: ANOVA, analysis of variance; IA, inhibitory avoidance; ICV, intracerebroventricular; mAChRs, muscarinic cholinergic receptors; PTZ, prenatal pentylenetetrazol; STL, step-through latency.

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whereas central muscarinic antagonist treatment was found to have the opposite effectance (Izquierdo et al., 1992). Studies have also shown that blockage of mAChR by scopolamine may impact recognition memory processes by affecting both recollection and familiarity (Mintzer and Griffiths, 2001, 2003; Sherman et al., 2003).

Prenatal pentylenetetrazol (PTZ)-induced kindling is an accepted animal model for the study of epilepsy and its consequences on memory (Chen et al., 2003). We have previously reported that prenatal PTZ-kindling induces passive avoidance learning deficits in male offspring, considering the important role of the mAChRs on learning and memory. It was logical to investigate the possible involvement of central mAChRs on learning and memory deficits induced by maternal PTZ-kindling, during pregnancy in adult male rats. For this purpose we examined effects of intracerebroventricular (ICV) administration of mAChRs' antagonist and agonist (scopolamine and pilocarpine, respectively) on passive-avoidance learning in pups born to kindled dams. This study for the first time, identifies a possible mechanism involved in learning deficit associated with maternal tonic-clonic seizures.

EXPERIMENTAL PROCEDURES

In vivo experiments

All procedures were performed in accordance with institutional guidelines for animal care and use which adhered to the international principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985). All efforts were made to minimize the number of animals used and their suffering. First part of experiments including animal selection, induction of epileptic seizures during pregnancy and selection of pups from each litter was performed as we have described previously (Pourmotabbed et al., 2011). Briefly, Male and female Wistar rats (Razi institute, Tehran, Iran) aged 3–4 months (250–300 g) were kept in animal facility room under standard condition and randomly paired. On embryonic day 13 (E13) (E0 being the day in which positive vaginal smear was observed), the pregnant dams were kindled by repetitive i.p. injection of PTZ (25 mg/kg body-weight, 1 ml/kg; Sigma, St. Louis, MO, USA), every 15 min until seizures occurred (two or three injections). Seizure activity was observed for 45 min and scored according to (Racine, 1972; Becker et al., 1992). Animals that reached stage 4 or 5 seizures were considered as the kindled rats. Control animals received normal saline. After parturition, pups were housed with their littermates until weaning. The male pups from each litter were housed singly in a cage. Pups from kindled and the control groups were then divided into ten subgroups of seven males each and were allowed to grow to 11 weeks of age.

Surgery and cannulation

After induction of anesthesia with ketamine (30 mg/kg, i.p.) and xylazine (2.5 mg/kg, i.p.), the rats were placed in the stereotaxic instrument, their heads were shaved and a guide cannula implanted 1 mm above the right cerebral ventricle using a 22-gauge needle. The task was carried out consistent with the atlas of Paxinos and Watson (Paxinos and Watson, 2006) (from bregma: anteroposterior, −1.0 mm; mediolateral, 1.6 mm; and −2.8 mm of the surface of skull). The cannula was fixed on the skull surface using dentistry cement and a glass screw. During the experiment, the drugs were injected through the guide cannula using 1-μl Hamilton syringe. In order to completely release the drug in the tissue spaces, the syringe was kept at the injection site for one min before removal.

Behavioral assessment

Step-through is a model of inhibitory avoidance (IA) task which is widely used in pharmacological studies of long-term memory in rodents (Izquierdo and McGaugh, 2000; Izquierdo et al., 2006).

Passive avoidance apparatus. The IA apparatus consisted of a Plexiglas box with two light (white) and dark (black) compartments of the same size ($20 \times 20 \times 30\ cm^3$) separated by a guillotine door that could be lifted manually. The floor of the dark compartment was made of stainless-steel grids (0.5-cm diameter) separated by a distance of 1 cm. Intermittent electric shocks (50 Hz, 3 s, 1 m A intensity) were delivered to the grids by an isolated stimulator (Jafari-Sabet, 2006)

Inhibitory-avoidance training. A week after surgery, a step-through passive avoidance test was performed as previously described (Pourmotabbed et al., 2011). All animals were allowed to become familiar with the experimental room for 1 h prior to each training or testing sessions. All sessions were carried out between 08:00 AM and noon. Each animal was gently placed in the light compartment for 20 s, after which the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency in entering into the dark (shock) compartment was recorded. Animals that had latency more than 100 s were excluded from the experiments. Once the animal crossed with all four paws to the dark compartment, the guillotine door was closed and the rat was taken out from the compartment (habituation trial). Thirty min later, the trial was repeated except that when the animal crossed to the dark compartment, the door was closed and a foot shock (50 Hz, 1 mA and 3 s) was immediately delivered to the grid floor of the dark compartment. After 20 s, the rat was removed from the apparatus and 2 min later the animal was retested. If the rat did not enter the shock compartment during 120 consecutive seconds, a successful acquisition of IA was recorded. Otherwise, the door was closed and the animal received the shock again. After retesting, if the rat learned IA response, it was removed from the apparatus and immediately received post-training injection of scopolamine (0, 0.5, 1, 2 or 3 μg/rat) or pilocarpine (0, 0.5, 0.75, 1 or 2 μg/rat) in sterile saline (Sigma, St. Louis, CA, USA) into the right cerebral ventricle.

Retention test. To examine long-term memory, the retention of the passive avoidance task was tested 24 h after the drug administration. Each animal was placed in