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Research report

NMDA receptor blockers prevents the facilitatory effects of post-training intra-dorsal hippocampal NMDA and physostigmine on memory retention of passive avoidance learning in rats

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Abstract

In the present study, the effects of post-training intra-dorsal hippocampal (intra-CA1) injection of an *N*-methyl-D-aspartate (NMDA) receptor agonist and competitive or noncompetitive antagonists, on memory retention of passive avoidance learning was measured in the presence and absence of physostigmine in rats. Intra-CA1 administration of lower doses of the NMDA receptor agonist NMDA (10^{-5} and 10^{-4} μg/rat) did not affect memory retention, although the higher doses of the drug (10^{-3}, 10^{-2} and 10^{-1} μg/rat) increased memory retention. The greatest response was obtained with 10^{-1} μg/rat of the drug. The different doses of the competitive NMDA receptor antagonist DL-AP5 (1, 3.2 and 10 μg/rat) and noncompetitive NMDA receptor antagonist MK-801 (0.5, 1 and 2 μg/rat) decreased memory retention in rats dose dependently. Both competitive and noncompetitive NMDA receptor antagonists reduced the effect of NMDA (10^{-2} μg/rat). In another series of experiments, intra-CA1 injection of physostigmine (2, 3 and 4 μg/rat) improved memory retention. Post-training co-administration of lower doses of NMDA (10^{-5} and 10^{-4} μg/rat) and physostigmine (1 μg/rat), doses which were ineffective when given alone, significantly improved the retention latency. The competitive and noncompetitive NMDA receptor antagonists, DL-AP5 and MK-801, decreased the effect of physostigmine (2 μg/rat). Atropine decreased memory retention by itself and potentiated the response to DL-AP5 and MK-801. In conclusion, it seems that both NMDA and cholinergic systems not only play a part in the modulation of memory in the dorsal hippocampus of rats but also have demonstrated a complex interaction as well.

Keywords: NMDA; MK-801; DL-AP5; Physostigmine; Passive avoidance learning; Retention; Dorsal hippocampus; Rat

1. Introduction

The amino acid glutamate is the principal excitatory neurotransmitter act, in the mammalian nervous system, upon receptors of the kainate, quisqualate and *N*-methyl-D-aspartate (NMDA) types [42,76]. NMDA glutamate receptors are widely distributed in the brain [48], but are present in high densities in the amygdala basolateral nuclei. The highest concentrations of NMDA binding sites are found in area CA1 of the hippocampus, with substantial concentrations also localized within the dentate gyrus [47].

NMDA receptors in hippocampal CA1 area are very important in the regulation of synaptic plasticity and the processes of learning and memory, including short- and long-term memory [35,36,50,51,54,83]. It has been suggested that memory improvement can be obtained by administration of NMDA receptor agonists or partial agonists into these and other structures in low doses that produce neither convulsions nor excitotoxic damage [18,19,52,59], while both competitive and noncompetitive NMDA receptor antagonists have been shown to impair learning and memory processes in various behavioral tasks [19,31,33,40,65,80]. Circumstantial evidence of the involvement of glutamatergic pathway derives from the well-known role of structures such as the hippocampus in learning and memory [64]. More specifically, lesions of certain glutamatergic pathways impair learning and memory [53].

Many clinical and experimental studies have shown that brain acetylcholine plays an important role in learning, memory and attention processes [16,20,57,68,78,79]. Much attention has been given to the role of acetylcholine in the hippocampal formation, which is an important neural substrate for cognitive functions. The entire hippocampal formation is innervated

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by cholinergic fibers derived from located in the medial septum/vertical limb of the diagonal band of Broca, which is a part of the septohippocampal projection [22,24,43]. A large number of studies have reported that muscarinic cholinergic agonists [8,15] and acetylcholinesterase inhibitors, which enhance the availability of acetylcholine in the synaptic cleft, improved memory [12,25,46,71,73,82], while anticholinergic drugs impair learning and memory in a variety of tasks [4,17,23,31,82]. Interactions between glutamatergic and cholinergic systems in learning and memory have been shown by several investigators [1,8,30,39,70]. Both muscarinic and nicotinic receptors activate glutamatergic pyramidal neurons and hence increase glutamate release [9,14].

Hippocampus, amygdala and septum operate in parallel in memory consolidation in the avoidance task [5,31]. The passive avoidance task has been used as a behavioral task to model learning and memory in a variety of experimental paradigms, including the study of different forms of cognition [72,74], cognition enhancers [55,67] and changes in septo-hippocampal neurons in the aged rat [37]. This task is the behavioral procedure of choice in many studies of learning and memory, probably because it requires very little specialist training of subjects and because the results are available quickly [77]. Most passive avoidance studies have utilized a paradigm in which animals are allowed to make an initial association between environment and a particular event (e.g. footshock) and are then tested 24 h later for retention of this initial association [3]. It has also been proposed that one-trial inhibitory training is a hippocampal-dependent task [60].

In order to investigate the interactions between NMDA receptors and the hippocampal cholinergic system, the effects of intra-dorsal hippocampal (intra-CA1) injection of NMDA receptor agonist and competitive or noncompetitive antagonists on the response induced by cholinergic drugs on passive-avoidance retention were evaluated in the present study.

2. Materials and methods

2.1. Animals

Male Wistar rats from Pasteur Institute (Iran), weighing 180–230 g at the time of surgery, were used. The animals were housed four per cage, in a room under a 12 h light:12 h dark cycle (lights on 07:00 h) and controlled temperature ($23 \pm 1\,^{\circ}C$). Animals had access to food and water ad libitum and were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Rats were handled about 3 min each day prior to behavioral testing. All experiments were performed between 9:00 and 13:00 h and each rat was tested only once. Eight animals were used in each group of experiments. All procedures in this study are in accordance with the guide for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of Faculty of Science, Tehran University (357: November 2000).

2.2. Passive avoidance apparatus

The passive avoidance apparatus consisted of two light (plexiglass) and dark (Black) compartments of the same size (20 cm × 20 cm × 40 cm) separated by a guillotine door (8 cm × 8 cm). 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, 5 s), 1.5 mA intensity, were delivered to the grid floor of the dark compartment by an isolated stimulator.

2.3. Stereotaxic surgery and microinjections

The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (4 mg/kg) and positioned in a Kopf stereotaxic instrument. Two 22-gauge stainless steel guide cannula as were placed (bilaterally) 2 mm above the intended site of injection, according to the atlas of Paxinos and Watson [62]. Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were −2.6 to −2.9 mm (depending on body weight) posterior to bregma, ±1.6–1.8 mm lateral to the midline and −2.5 to −2.8 mm ventral of the dorsal surface of the skull. Cannula as were secured to anchor jewellers' screws with dental acrylic. To prevent clogging, stainless steel stylets (27 gauge) were placed in the guide cannula until the animals were given the CA1 injection. All animals were allowed 1 week to recover from surgery and anesthesia.

The animals were gently restrained by hand, and the stylets were removed from the guide cannula and replaced by 27-gauge injection needles (2 mm below the tip of the guide cannula). Each injection unit was connected by polyethylene tubing to a 1-μl Hamilton syringe. The left and right CA1 were infused with 0.5 μl solution on each side (1 μl/rat) over a 60-s period. The injection needles were left in place for an additional 60 s to allow diffusion and then the stylets were reinserted into the guide cannulas.

2.4. Behavioral testing

2.4.1. One trial inhibitory-avoidance training

The rats were allowed to habituate to the laboratory environment 1 h before each of the training or testing sessions. All training and testing was carried out between 08:00 and 12:00 h. Each animal was gently placed in the light compartment for 10 s, after which the guillotine door was raised and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. If an animal waited more than 100 s to cross to the other side, it was removed from the experiment. Once the animal crossed with all four paws to the next compartment, the door was closed and a 1.5 mA foot shock was delivered for 5 s. The rat was then removed from the apparatus and immediately given an intra-dorsal hippocampal (intra-CA1) injection via the guide cannula.

2.4.2. Retention test

Twenty-four hours after training, a retention test was performed to determine long-term memory. Each animal was placed in the light compartment for 10 s, the door was opened, and the latency for entering into the shock compartment (as described in the training session) was measured. The test session ended when the animal entered the shock compartment or remained in the light compartment for 600 s (criterion for retention). During these sessions, no electric shock was applied.

2.5. Drugs

The drugs used in the present study were *N*-methyl-D-aspartate, D,L-2-amino-5-phosphonopentanoic acid (DL-AP5), (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cycloheptan-5,10-imine maleate ((+)-MK-801 maleate) (Tocris Cookson Ltd., UK), atropine sulphate (Merck, Germany), and physostigmine salicylate (Sigma, Poole, UK). All drugs were dissolved in sterile 0.9% saline and were injected into the intra-dorsal hippocampal (intra-CA1) in a volume of 1 μl/rat. Methylene blue (1 μl/rat) injected in a number of animals ($n = 8$) was found not to spread beyond the CA1 region of dorsal hippocampus, indicating that the volume of injection was suitable for drug administration.

2.6. Verification of cannulae placements

After completion of the experimental sessions, each animal was killed with an overdose of chloroform. Animals received a 0.5-μl/side injection of ink (1% aqueous methylene blue solution). The brains were then removed and fixed in a 10% formalin solution for 10 days before sectioning. The fixed brains were then sliced directly across the injection sites, and the cannula placements were verified using the atlas of Paxinos and Watson [62]. The results