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Effects of cholinergic system of dorsal hippocampus of rats on MK-801 induced anxiolytic-like behavior

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ABSTRACT

Rationale: Some investigations have shown that the glutamate receptors play a critical role in cognitive processes such as learning and anxiety. *Objectives*: The possible involvement of the cholinergic system of the dorsal hippocampus in the anxiolytic-like response induced by MK-801, NMDA receptor antagonist, was investigated in the present study. *Methods*: Male Wistar rats were used in the elevated plus maze apparatus to test the parameters: open arm time (%OAT), open arm entries (%OAE), close arm time (%CAT), close arm entries (%CAE) and other exploratory behaviors (locomotor activity, grooming, rearing and defecation) of anxiety-like response. *Results*: The data indicated that intra-CA1 administration of MK-801 increased %OAT (2 μg/rat) and %OAE (1 and 2 μg/rat) while decreased %CAT and %CAE and did not alter other exploratory behaviors, indicating an anxiolytic-like effect. Moreover, intra-hippocampal injections of mecamylamine, a cholinergic receptor antagonists (2 μg/rat) and scopolamine (4 μg/rat), by themselves, 5 min before testing, increased %OAT and %OAE but decreased %CAT and %CAE and did not alter locomotor activity and other exploratory behaviors, suggesting an anxiolytic-like effect. On the other hand, intra-CA1 co-administration of an ineffective dose of scopolamine (3 μg/rat), but not mecamylamine (1 μg/rat), with an ineffective dose of MK-801 (0.5 μg/rat) increased %OAT and %OAE and decreased %CAT and %CAE. The data may indicate the possible involvement of the cholinergic system of the CA1 in the anxiolytic-like response induced by MK-801.

Glutamate is a major excitatory amino acid neurotransmitter in the central nervous system. Different pharmacological actions of glutamate, which has a critical role in physiologic or pathologic conditions, depend on the specific synapse receptor expression [1]. Glutamate exerts its effects through its ionotropic and metabotropic receptors. Ionotropic glutamate receptors are comprised of NMDA, AMPA and kainite. These receptor sites are generally postsynaptic and mediate fast excitations and synaptic plasticity associated with the opening of sodium- and calcium-permeable ligand-gated ion channels. Metabotropic glutamate receptors, on the other hand, are present in the presynaptic, postsynaptic, glial and heterosynaptic sites. They modulate postsynaptic excitability and provide positive or negative feedback to modulate the further release of neurotransmitters [10]. Both ionotropic NMDA and AMPA receptors have a role in the maintenance of cognitive functions including learning, memory and attention. Some investigations have also indicated that MK-801, a non-competitive channel blocker and an NMDA receptor antagonist, is an effective anxiolytic agent in the EPM [5].

It has also been revealed that the cholinergic system plays an essential role in anxiogenic-like response [16], learning and memory [1]. On the other hand, to date, two nicotinic (nAChRs) and muscarinic (mAChRs) receptors have been identified [1] both of which are involved in anxiety-like behaviors [6]. Some studies have indicated that cholinergic activation induces anxiolytic-like behavior [3] while both nicotinic or muscarinic receptor antagonists induce anxiogenic-like behavior [7].

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The interactions between the cholinergic and glutamatergic neurotransmitter systems are not yet fully understood. There is, however, a report that the hippocampal formation receives dense innervations of cholinergic afferents from the medial septum and diagonal band of Broca [1]. There is also a glutamatergic hippocampo-septal pathway arising from the pyramidal neurons of the Amon's horn and projecting to the lateral septum [14]. A study showed that intra-hippocampal injection of NMDA agonist led to decrease in acetylcholine output [14]. Therefore, the aim of present study, on the basis of the findings of the previous ones, is to investigate the possible involvement of nAChR and mAChR cholinergic receptors of the dorsal hippocampus (CA1) in the anxiolytic-like effect of MK-801 in the elevated plus maze (EPM) task. EPM has been used as a valid method for measuring anxiety-like behavior in a number of investigations [3,17].

Male Wistar rats that were bred in an animal house in the department of pharmacology (Tehran University of Medical Sciences, Iran), weighing 200–220 g at the beginning of study, were housed four per cage in a temperature-controlled ($22 \pm 2\,^{\circ}C$) room with a 12 h light/dark cycle (lights on 08:00 h) and relative humidity of 45–55% with access to food and water ad libitum. The animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Each rat was handled for about 3 min each day prior to behavioral testing. All experiments were performed between 9:00 h and 12:00 h and each rat was tested only once. Eight animals were used in each group of experiments. The study was approved by the Ethics Committee of the Tehran University of Medical Sciences which corresponds to the national guidelines for animal care and use.

Rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) and were placed in a stereotaxic instrument (Stoelting Co., IL, USA). The stainless steel guide cannulae (22-gauge, Supa Medical Devices, Tehran, Iran) were implanted bilaterally into the right and left dorsal hipoccampus (CA1) according to Paxinos and Watson [13]. Stereotaxic coordinates for the CA1 regions of dorsal hypocampi were incisor bar (−3.3 mm), −3 to −3.5 mm (depending on body weight) posterior to bregma, ±1.8–2 mm lateral to the sagital suture and −2.8 to −3 mm ventral of the dorsal surface of the skull. Injections were performed by means of an internal cannulae (27-gauge, Supa Medical Devices, Tehran, Iran), terminating 1 mm below the tip of the guides, connected by polyethylene tubing to a 1-μl Hamilton syringe. All intra-CA1 injections were bilateral with a 0.5 μl solution on each side (1 μl/rat) over a 60-s period. The inner cannulae were left in place for an additional 60 s to allow diffusion of the solution and to reduce the possibility of reflux.

The maze consisted of four arms. Two of the arms had no side or end walls (open arms; 50 cm × 10 cm); the other two had side walls and end walls but were open on the top (closed arms; 50 cm × 10 cm × 40 cm). Where the four arms intersected, there was a square platform of 10 cm × 10 cm. The maze was elevated to a height of 50 cm. In order to test total arm entries on the maze, rats were placed in a wooden test arena (50 cm × 50 cm × 35 cm) for 5 min prior to maze testing. Five days after implantation, the effects of intra-CA1 injection of drugs were tested in the elevated plus maze. Rats were placed in the experimental room at least 1 h before testing. For testing anxiety-like behaviors, the rats were individually placed in the center of the maze facing a closed arm and allowed 5 min of free exploration. During this 5 min, the percentage of open arm times, open arm entries, close arm times and close arm entries as the standard anxiety indices were calculated as follows: (a) % OAT (the ratio of time spent in the open arms to total time spent in any arms × 100); (b) %OAE (the ratio of entries into open arms to total entries × 100); (c) %CAT (the ratio of time spent in the close arms to total time spent in any arms × 100); (D) %CAE (the ratio of entries into close arms to total entries × 100). Total arm entries were measured as a relative pure index of locomotor activity [17]. These behaviors with numbers of rearings, groomings and defecations were recorded by a video camera while a monitor and a computer-recording system were installed in an adjacent room. Raw data were used to manually calculate the anxiety-like behaviors. Entry was defined as all four paws in the arms and measured by a hand counter. Experiments were performed by someone blind to doses of drugs and statistical results. For none of the experiments the data of rearing, grooming and defecation are shown.

For surgical procedure, ketamine and xylazine were used (Alfasan Chemical Co., Woerden, and Holland). MK-801 (Sigma, Poole, Dorset, UK), mecamylamine and scopolamine (Sigma Chemical Co., St. Louis, CA, USA) were used for intra-CA1 injection. The drugs were dissolved in sterile 0.9% saline just before the experiment. Control animals received saline. The doses of drugs and the time interval between drug infusions and behavioral testing were based on previous work [1] or preliminary analyses.

Five groups of rats underwent surgery and received saline (1 μl/rat) or MK-801 (0.5, 1 and 2 μg/rat) while sham operated group did not receive anything. The test session was held 5 min after drug injection. %OAT, %OAE, %CAT, %CAE, locomotor activity, grooming, rearing and defecation were measured as described.

In these experiments, seven groups of animals received saline (1 μl/rat, intra-CA1) or nicotinic receptor antagonist, mecamylamine (0.5, 1 and 2, μg/rat, intra-CA1), 5 min before the injection of saline (1 μl/rat, intra-CA1) or an ineffective dose of MK-801 (0.5 μg/rat, intra-CA1). The test session was held 5 min after the injection of the drugs.

In these experiments, seven groups of animals received saline (1 μl/rat, intra-CA1) or muscarinic receptor antagonist, scopolamine (2, 3 and 4, μg/rat, intra-CA1) 5 min before the injection of saline (1 μl/rat, intra-CA1), or MK-801 (0.5 μg/rat, intra-CA1). The test session was held 5 min after the injection of the drugs.

One-way ANOVA was run for comparing the effects of the drugs. Following a significant *F*-value, post-hoc analysis (Tukey-test) was performed for assessing specific inter-group variations. Differences with $P < 0.05$ between experimental groups at each point were considered statistically significant.

Following behavioral testing, the rats were deeply anesthetized and received 1 μl of a 4% methylene-blue solution bilaterally into the CA1 (0.5 μl/side). Each animal was decapitated and its brain was removed and placed in formaldehyde (10%) for seven days before sectioning. Finally, the brains were sliced and the sites of injections were verified according to Paxinos and Watson [13]. Data from animals with injection sites located outside the CA1 regions were discarded.

Cannulae were implanted into the CA1 regions of dorsal hippocampus of a total of 173 animals, but only data from 152 animals with correct cannulae implants were included in the statistical analyses (Fig. 1).

Fig. 2 shows the effects of MK-801 in the elevated plus-maze. One-way ANOVA and post hoc analysis revealed that MK-801 (1 and 2 μg/rat) increased %OAT [$F(4, 35) = 6.24$, $P < 0.001$] and %OAE [$F(4, 35) = 12.23$, $P < 0.001$] and decreased %CAT [$F(4, 35) = 5.57$, $P < 0.05$] and %CAE [$F(4, 35) = 5.43$, $P < 0.01$] but did not alter locomotor activity [$F(4, 35) = 1.07$, $P > 0.05$], grooming [$F(4, 35) = 1.86$, $P > 0.05$], rearing [$F(4, 35) = 0.65$, $P > 0.05$] and defecation [$F(4, 35) = 0.48$, $P > 0.05$]. This indicates that MK-801 induced anxiolytic-like response.

Analyses revealed that mecamylamine (2 μg/rat) increased %OAT [$F(3, 28) = 11.43$, $P < 0.001$] and %OAE [$F(3, 28) = 5.87$, $P < 0.001$] and decreased %CAT [$F(3, 28) = 12.3$, $P < 0.001$] and %CAE [$F(3, 28) = 14.7$, $P < 0.001$] but did not alter locomotor activity [$F(3, 28) = 3.73$, $P > 0.05$], grooming [$F(3, 28) = 1.26$, $P > 0.05$], rearing [$F(3, 28) = 0.24$, $P > 0.05$] and defecation [$F(3, 28) = 3.63$, $P > 0.05$],