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Original article

Microinjection of NMDA Receptor Agents into the Central Nucleus of the Amygdale Alters Water Intake in Rats

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Abstract

Objective(s)

The central nucleus of the amygdala (CeA) is a forebrain structure which is important in regulation of ingestive behavior and there is direct and circumstantial evidence to indicate that some circuits involved with feeding behavior include glutamatergic elements. The present study examined whether administration of NMA (N-Methyl-DL-aspartic acid) or MK801 into the CeA altered water intake under deprivation.

Materials and Methods

Animals were deprived for 24 hr before tested for water intake. NMDA (N-methyl-D-aspartate) glutamatergic receptor agonist, NMA and its antagonist, MK801 were infused bilaterally, and water intake measured for 1 hr thereafter.

Results

The intra-CeA injection of NMDA glutamatergic agonist, NMA (0.25, 0.5 and 0.75 μg/rat) increased water intake (P<0.05). However, administration of NMDA glutamatergic antagonist, MK801 (0.25, 0.5 and 1 μg/rat) decreased water intake significantly (P<0.05).

Conclusion

These data suggest that NMDA receptors in the CeA are responsible for the glutamatergic modulation of water intake in this nucleus.

Keywords: Central amygdala, N-methyl-D-aspartate, Water intake

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Introduction

Drinking and electrolyte balance are regulated by a complex network of brain regions and diverse neurochemical mechanisms, including those of the glutamatergic system. A great deal of evidence indicates that the limbic areas such as amygdala plays an important role in the control of food and water intake (1).

Glutamatergic mechanisms are present in all brain regions regulating feeding, including the nucleus accumbens, hypothalamus, and amygdala. A limbic forebrain region strongly implicated in the motivational mechanisms for feeding (2).

Ionotropic glutamatergic receptors can be subdivided into N-methyl-D-aspartate (NMDA), (3) kaı¨nate and quisqualate receptors named after the agonists that selectively bind to these receptors. These synthetic selective agonists resemble either glutamate or aspartate (4-5).

The NMDA receptor-channel complex has several characteristic features. There are several regulatory sites on this NMDA receptor complex. Three of these modulatory sites are outside the ion channel (the neurotransmitter glycine site, the polyamine site and the zinc site) and are excitatory in nature. The inhibitory modulator sites are located inside the ion channel. Precise modulation is required for normal neuronal functioning, depolarisation of the NMDA receptor results in a slow rising, long lasting current (4).

In most CNS synapses, NMDA receptors coexist with either AMPA or kaı¨nate receptors. These latter receptors are thought to be involved in amplification of the glutamate signal. The level of concurrent depolarization depends on AMPA/kaı¨nate activation and other modulator signals (4).

Previous studies showed that injections of the excitatory amino acid glutamate into the limbic area such as lateral hypothalamus are able to induce robust feeding and drinking (1). Systemic, intracerebroventricularly (ICV) or local administration of Glu or Glu agonists into the lateral hypothalamus can evoke a dose-related stimulation of food intake in mammals that can be mediated by NMDA as well as by other excitatory amino acid (EAA) receptor subtypes. However, systemic ICV and local injections of a number of EAA receptor antagonists into the median raphe or into the nucleus accumbens can elicit increases in food intake, indicating that eating behavior can be modulated by multiple EAA-mediated circuits located both centrally and at the periphery (6).

No data on the role of central amygdale glutamatergic circuits in the control of feeding behavior in animal forms are available in the literature. In this study the effect of central amygdala NMDA receptor agents on the modulation of water intake was investigated.

Materials and Methods

Animals

Male wistar rats from Pasteur Institute (Iran), weighing 180–230 g at the time of surgery, were used. Animals were housed four per cage in a room with a 12:12 hr light / dark cycle (lights on 07:00 hr) and controlled temperature (23±1 °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 behavioural testing.

Drugs

The drugs used in the present study were NMA (N-Methyl-DL-aspartic acid) and MK801 or Dizocilpine hydrogen maleate (5-methyl-10, 11- dihydro-*5H*-dibenzo cyclohepten-5, 10-imine maleate) (Sigma Chemical Co., USA).

Stereotaxic surgery and microinjections

Rats were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) and placed in a Stoelting stereotaxic instrument. The stainless steel guide cannula (22-gauge) was implanted in the right and left CeA regions according to Paxinos and Watson (7). Stereotaxic coordinates for the CeA regions were: − 2.3 mm posterior to bregma, ±4.1 mm lateral to the midline and − 7.2 mm ventral of the dorsal surface of the skull. The cannula was fixed to the skull with acrylic dental cement. The animals were allowed 5 days before the

test to recover from surgery. The left and right CeA were infused by means of an internal cannula (27-gauge), terminating 1 mm below the tip of the guides, connected by polyethylene tubing to a 1-µl Hamilton syringe. On each side 0.5 µl solution was injected (1 µl/rat) over a 60 Sec period. The inner cannula was left in place for an additional 60 Sec to allow diffusion of the solution and to reduce the possibility of reflux. Intra-CeA injections were made 5 min before testing (8).

Water intake experiments

Rats had free access to water and food and were put in the separate metabolic cages at least 7 days before the experiments began. The amount of water ingested in the various experiments was measured with 0.1 ml-graduated glass burettes adapted with a metal drinking spout. Intake was induced by water deprivation during the 24 hr that preceded the experiment (9).

Experiment 1: Four groups of animals, deprived of water, three groups intra-CeA injected with NMA (0.25, 0.5 and 0.75 µg/rat; 1 µl/rat; 0.5 µl/rat in each side) and other group received equivalent volume of saline. Immediately after injection each rat was returned to its cage and we measured the cumulative water intake for 60 min after injection of the solutions.

Experiment 2: Four groups of animals, deprived of water, three groups intra-CeA injected with MK801 (0.25, 0.5 and 1 µg/rat; 1 µl/rat; 0.5 µl/rat in each side) and other group received equivalent volume of saline. Immediately after injection each rat was returned to its cage and we measured the cumulative water intake for 60 min after injection of the solutions.

The numbers of rats were six in each group and experiments were done after dark onset.

Statistical analysis

Since data displayed normality of distribution and homogeneity of variance, one-way ANOVA was used for comparison between the effects of different doses of drugs with vehicle.

Results

The results showed that intra CeA injection of NMDA glutamate receptor NMA (0.25 and 0.5 µg/rat) increase water intake (P<0.05) (Figure 1).

Intra-CeA injection of NMDA receptor antagonist, MK801 at the doses of 0.25, 0.5 and 1 µg/rat decreases cumulative water intake in male wistar rats (P<0.5) (Figure 2).

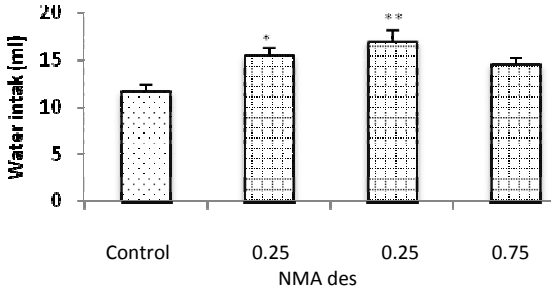

Figure 1. Effects of intra-CeA injection of NMA (0.25, 0.5 and 0.75 µg/rat) or saline on cumulative water intake in water deprived rats (24 hr), 60 min after injection of solutions. Data for water intake are expressed as the mean±SEM (n= 6).*P<0.05, **P<0.01 compared with saline-injected rats (One-way ANOVA).

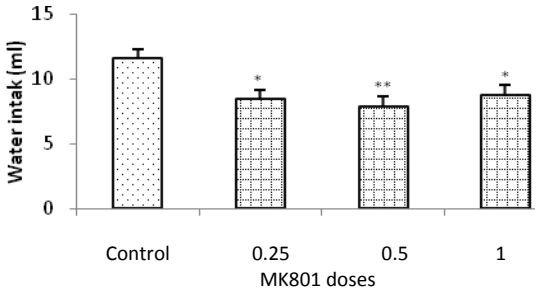

Figure 2. Effects of intra-CeA injection of MK801 (0.25, 0.5 and 1 µg/rat) or saline on cumulative water intake in water deprived rats (24 hr), 60 min after injection of solutions. Data for water intake are expressed as the mean±SEM (n= 6).*P<0.05, **P<0.01 compared with saline-injected rats (One-way ANOVA).

Discussion

Results of present study show that central amygdala NMDA receptor modulates the drinking behavior of rats. Some studies have shown that NMDA-glutamatergic system has a role on modulation of feeding behaviors. For example systemic injection of the non-competitive NMDA antagonist, MK801, increased food intake in rats (10). These

results differ from our results, in which MK-801 decreased water intake when injected to CeA. The neuroanatomical site(s) of action that through which MK-801 increases feeding is unknown. The potential exists for NMDA receptor involvement in the control of food intake at several levels of the central and peripheral nervous system (11). Intake of both food and water can be elicited in non-deprived rats by the local administration of NMDA receptor antagonists into the median raphe (12, 13). Our results showed that intra-CeA injection of NMA increased water intake in rats. There is a little published information about the effects of NMA on drinking behavior. However, excitatory amino acids (EAA), such as glutamate and its agonists such as NMA have been described in brain areas that are implicated in the control of ingestive behavior. For example, Treece *et al* demonstrated robust increases in food intake following blocking of NMA binding site or inhibition of glutamatergic neurons in the nucleus tractus solitarii (NTS) (14). Areas of the hypothalamus, associated with the control of food and water intake, express mRNA for NMDA glutamate receptors (15). Presentation of highly palatable solid or liquid foods to food-deprived rats resulted in an immediate increase in glutamate output of more than 200% over baseline in the lateral hypothalamus (15).

Conclusion

Our study shows that activation of NMDA-glutamatergic system of central amygdala alters water intake in rats. However, more studies are necessary for demonstration of mechanisms that under which this system can affect feeding behaviors.

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