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

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Abstract

Food intake is the primary factor for body weight regulation in animals. Eating behavior is known to be modulated by various neurotransmitters and receptors. The central nucleus of the amygdala (CeA) is the forebrain structures which are important in regulation of ingestive behavior. 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 or MK801 in the CeA alters food intake under deprivation. Animals were deprived for 24 h before tested for food intake. NMDA glutamatergic receptor agonist, NMA (N-Methyl-DL-aspartic acid) and its antagonist, MK801 (Dizocilpine hydrogen maleate) were infused bilaterally, and food intakes were measured for 12 h post-injection. The intra-CeA injection of NMDA glutamatergic agonist NMA (0.25, 0.5 and 0.75 µg/rat) showed no effect on cumulative food intake. However, administration of NMDA glutamatergic antagonist, MK801 (0.25, 0.5 and 1 µg/rat) increased food intake in the deprived rats. The finding suggest that NMDA receptors in the CeA are responsible for the glutamatergic modulation of feeding in this nucleus.

Keywords: Food intake, NMDA glutamatergic antagonist, MK801.

Introduction

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].

For years, the main effects of glutamate were thought to be exclusively mediated by ion channel mechanisms. However, glutamate receptors can now be categorised into two major groups; (I) ionotropic and (II) metabotropic receptors. This categorisation is based on intracellular/extracellular coupling and on different pharmacological and biochemical characteristics. Ionotropic receptors

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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. The quisqualate receptor has been renamed as the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor [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 and depolarisation of the NMDA receptor results in slow rising, long lasting current [3, 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 depolarisation 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 [1, 6]. Similar effects can be observed following injections of glutamate antagonists [7].

In this study the effect of central amygdale NMDA receptor agents on the modulation of food intake is investigated.

Experimental

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 h light / dark cycle (lights on 07:00 hours) 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. All experiments were performed after dark onset and each rat was tested only once. Six animals were used in each experiment.

Drugs

The drugs used in the present study were NMA (N-Methyl-DL-aspartic acid) and MK801or Dizocilpine hydrogen maleate (5-methyl-10,11- dihydro-5H-dibenzo cyclohepten-5,10-imine maleate) (Sigma Chemical Co., USA).

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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 [8]. 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 s period. The inner cannula was left in place for an additional 60 s to allow diffusion of the solution and to reduce the possibility of reflux. Intra-CeA injections were made 5 min before testing [9].

Food intake experiments

Rats had free access to water and food and were left in the seprate metabolic cages at least 7 days before the experiments began. In this experiment, the groups of animals after 1 week of habituation to their new housing conditions, deprived of food for 24 h (rats were fasted in separate cages with free access to water) and after fasting period divided to equal groups and injected with drugs. Control group received equivalent volume of saline.

Experiment 1: Four groups of animals, deprived of food, three groups intra-CeA injected with NMA (0.25, 0.5 and 0.75 µg/rat; 1 µl/rat; 0.5µl/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 food intake for 12 h post injection.

Experiment 2: Four groups of animals, deprived of food, 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 food intake for 12 h post injection.

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.

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Results

One way-ANOVA revealed that intra CeA injection of NMDA glutamate receptore NMA (0.25 and 0.5 µg/rat) caused no singnificant effects on cumulative food intake (Figure 1).

Intar-CeA injection of NMDA receptor antagonist, MK801at the doses of 0.25, 0.5 and 1 µg/rat increased cumulative food intake in food deprived rats ($p<0.5$) (Figure 2).

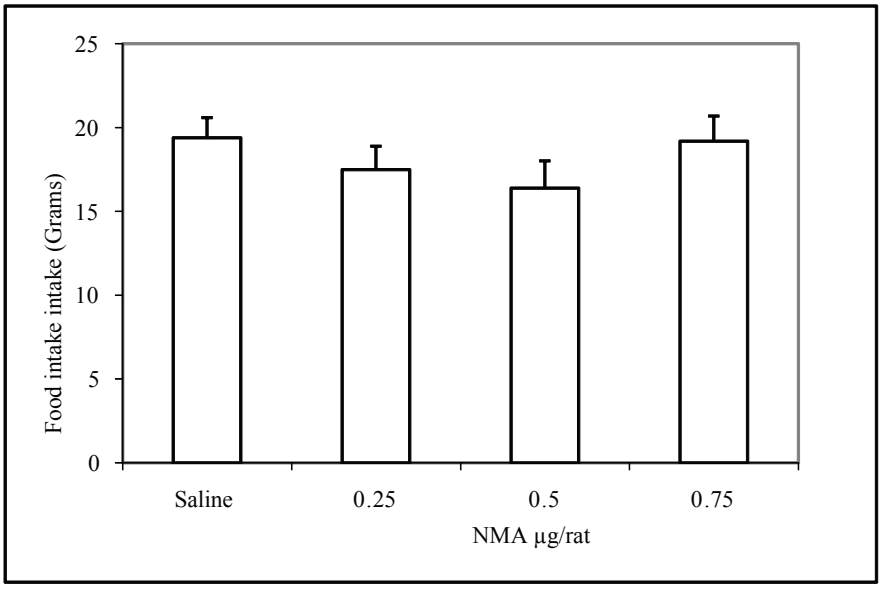

Figure 1. Effects of inta-CeA injection of NMA(0.25, 0.5 and 0.75 µg/rat) or saline on cumulative food intake in food deprived rats (24 h), 12 h after injection of solutions. Data for food intake are expressed as the mean ± SEM (n = 6).

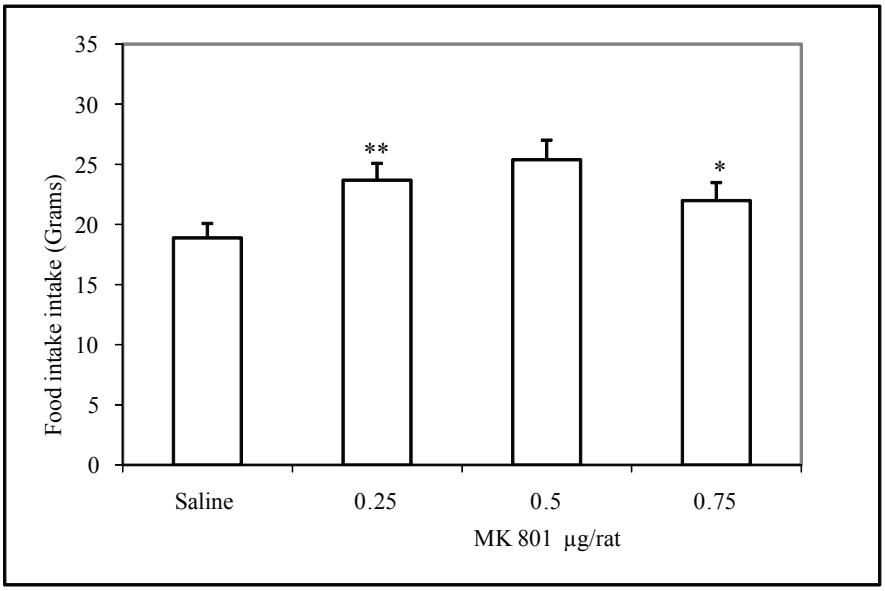

Figure 1. Effects of inta-CeA injection of MK801(0.25, 0.5 and 1 µg/rat) or saline on cumulative food intake in food deprived rats (24 h), 12 h post-injection. Data for food intake are expressed as the mean ± SEM (n = 6).*P<0.05, **P<0.01 compared with saline-injected rats.

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Discussion

The present study examined the possible involvement of central amygdale glutamatergic circuits in food intake. Results showed that central amygdale NMDA receptors affect the modulation of feeding behavior in rats. Previous investigation demonstrated that glutamatergic system of the brain have an important role in the modulation of ingestive behaviors. Our result indicated that Mk-801 increases food intake. Studies showed that NMDA glutamatergic antagonists elicit feeding behaviors, for example systemic injection of the non-competitive NMDA antagonist, MK801, increased food intake in rats [10]. However, the neuroanatomical site(s) of action through which MK-801 increases feeding is unknown. The potential for NMDA receptor involvement in the control of food intake aexists several levels of the central and peripheral nervous system [7]. 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 [11, 12]. Therefore, while the median raphe is the site that merits further analysis with regard to NMDA receptor participation in control of food intake, it would be premature to conclude that the effects observed after peripheral MK-801 administration can be referred to an action in the raphe. Excitatory amino acids (EAA), such as glutamate and its agonists, or their binding sites have been described in brain areas that are implicated in the control of food intake. For example, neurons or terminals in the nucleus tractus solitarii (NTS) release glutamate [13, 14]. Areas of the hypothalamus, associated with the control of food intake, express mRNA for ionotropic glutamate receptors [15]. In addition, mRNA coding for the R1 subunit of the N-methyl-d-aspartate (NMDA) receptor has been localized to intrinsic neurons of the stomach and small intestine [16]. This study showed that inhibition of NMDA-glutamatergic system of central amygdale increases food intake in rats.

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