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Research report

Effects of glutamate and its metabotropic receptors class 1 antagonist in appetitive taste memory formation

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HIGHLIGHTS

- The role of cortical glutamatergic system in attenuation of neophobia was studied.
- Glutamate could act, at least in part, as a visceral signal of poisoning in the IC.
- AIDA infusion into the IC produces acceleration in attenuation of neophobia.
- Cortical glutamate, through its mGluR, modulates the consumption of familiar taste.

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ABSTRACT

Cortical glutamatergic activity is known to be important for memory formation in different learning tasks. For example, glutamate activity in the insular cortex plays an important role in aversive taste memory formation by signaling the unconditioned stimulus. However, the role of glutamate in the insular cortex in appetitive taste learning has remained poorly studied. Therefore, we considered the function of glutamate in attenuation of neophobia, a model of appetitive taste recognition memory. For this purpose, we performed infusions of vehicle, glutamate, a specific mGluR1 antagonist (AIDA) or a combination of glutamate and AIDA at 0 or 30 min, and glutamate or vehicle at 60 min after novel saccharin consumption. Glutamate infusion impaired appetitive taste recognition memory when infused at 0 or 30 min, whereas, AIDA infusions produced enhanced appetitive memory at the same infusion times. Furthermore, when glutamate and AIDA were infused together no effect on attenuation of neophobia was observed. As opposed to shorter infusion times, the administration of glutamate 60 min after the presentation of the saccharin consumption was ineffective in the impairment of the appetitive taste memory. These results are discussed in view of the effect of glutamate and its mGluR1 during the appetitive taste recognition memory formation in the insular cortex.

1. Introduction

Taste recognition memory is a highly adaptive type of associative learning that allows an animal to remember a flavor and to establish a relationship of this stimulus with its post-ingestional consequences, leading to the formation of either an appetitive or aversive taste memory that determines a subsequent consumption or rejection of the taste [3]. Thus, when a novel taste is followed by malaise, it is recognized as an aversive signal and the animal will reject that flavor (conditioned taste aversion). On the other hand, if the novel taste does not have any aversive consequence it becomes recognized as an appetitive signal and the animal will consume that taste in following exposures to the stimulus (attenuation of neophobia). Besides, its adaptative relevance, taste memory is also a model frequently used to study the neural mechanisms underlying learning and memory, partly because it can be efficiently acquired in a single trial [3,4,20].

Both aversive and appetitive taste memories depend on a neural representation of the taste, which is possibly stored in parallel in several brain regions [3]. Among the brain structures involved in taste memory formation, insular cortex (IC) plays a crucial role processing taste signals during the formation of either appetitive or aversive taste memory [3,10,20].

Concerning the neurotransmission associated to memory formation, glutamatergic transmission has been implicated in

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different types of learning [1,5,21,24,28]. Glutamate receptors have been classified as ionotropic or metabotropic (mGluR) receptors. The ionotropic receptors are the NMDA, AMPA and kainate receptors, while the mGluR can be subdivided into three groups: group I includes mGluR1 and mGluR5; group II includes mGluR2 and mGluR3; and group III includes mGluR4, mGluR6, mGluR7 and mGluR8 [16].

More specifically to taste memory, glutamatergic activity in the IC through N-methyl-D-aspartate receptor (NMDAr) activation, has been shown to be determinant in the association of a taste with gastric malaise in the formation of an aversive taste memory [8], while NMDArs are less important for appetitive taste memory formation [22] observed through attenuation of neophobia learning [13]. However, antagonists of both NMDA and metabotropic glutamate receptors produce an impairment of latent inhibition, a type of appetitive taste memory wherein a weak conditioned taste aversion (CTA) is acquired when the taste has been previously presented without malaise [2].

It has been reported that the infusion of a mGluR5 antagonist injected directly into the IC or in the basolateral amygdala does not affect CTA memory formation but rather delays its extinction, suggesting a strengthening of this memory [27]. Moreover, it has been reported that i.p. injection of an mGlutR5 antagonist or mGluR antagonist (MCPG) infused into the IC impairs latent inhibition for CTA [2,6]. Although, the mGluR5 have been reported to play a role during CTA memory formation [9,26] the precise role of the different mGluR subtypes on appetitive taste memory formation in the insular cortex have not yet been elucidated.

Despite the large number of reports about the role of the glutametergic system in aversive taste memory, there are few investigations about its importance in appetitive taste memory formation. Thus, we took the endeavor to determine the role of glutamatergic system in the IC during the formation of appetitive taste memory in attenuation of neophobia, exploring the possible role of type I mGluR.

2. Materials and methods

2.1. Animals

Male Wistar rats weighting between 280 and 320 g at the time of surgery were used. They were individually caged and kept in a 12 h light:12 h dark cycle. All behavioral manipulations were performed in the light cycle phase. Rats received food and water *ad libitum* unless otherwise indicated. Experiments were performed in accordance with the Rules in Health Matters (Ministry of Health, Mexico) and with approval of the local Animal Care Committee.

2.2. Surgery

Animals were implanted bilaterally with 12-mm 23-gauge stainless steel cannulae under anesthesia (sodium pentobarbital, 40 mg/kg, i.p.) using standard stereotaxic procedures. The tips of the cannulae were aimed 2.5 mm above the IC (anteroposterior, +1.2 mm; lateral, ±5; ventral, −3.9 mm; from Bregma) [23]. Cannulae were fixed to the skull with dental acrylic cement and anchored with two surgical screws placed in the skull. Stylets were inserted into the guide cannulae to prevent clogging.

2.3. Drugs and infusion procedures

Artificial Cerebrospinal Fluid (ACSF) solution (118 mM NaCl, 19 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 2.5 mM $CaCl_2$, pH 7.4; T.J. Baker, Xalostoc, Mexico; 3.3 mM glucose, Sigma, St. Louis, MO) was used as vehicle. L-Glutamate (Sigma) and 1-aminoindan-1,5-dicarboxylic acid (AIDA, Tocris) were dissolved the administration day, according to producers instruction. The dose of 21.37 mM of glutamate was chosen on basis of previous data [19]. The doses of AIDA reported previously in intracerebral infusions range from 40 mM [18] to 0.05 mM [25]. Since there is a huge range of reported doses used of AIDA and some of them were used by i.p. injection, we performed a dose–response curve with three different doses of AIDA, 3.5 mM; 7.04 mM and 10.5 mM (data no shown) using intra cortical infusions.

Intracortical microinjections were given to hand restrained, conscious animals. Stylets were withdrawn from the guide cannulae and injection needles (30 gauges) were inserted, extending 2.5 mm from the tip of the guide cannula in order to reach the IC. Injection needles were connected *via* polyethylene tubing to 10 μL Hamilton micro syringes driven by an automated micro infusion pump (Carnegie Medicine, Stockholm, Sweden). A total volume of 0.5 μL/min per hemisphere was delivered in IC in one minute. After the injections, needles were retained in the guide cannulae for another 1-min to allow diffusion of the solution into the tissue and to avoid reflux along the injection track.

Different cohorts of animals were infused with ACSF (VEH) or Glutamate (GLUT) at 0, 30 and 60 min after novel saccharin consumption. Other groups of animals received injections of vehicle, AIDA (AIDA) or a cocktail of glutamate and AIDA (GLUT&AIDA) at 0 or 30 min after novel saccharin presentation. We performed the infusions in the AIDA and GLUT&AIDA groups when the infusion of glutamate alone caused an effect upon appetitive taste memory.

2.4. Behavioral procedures

After surgery, animals received food and water *ad libitum* for five days, and the next day were water deprived for 24 h. All the animals were handled every day in order to diminish the stress by the microinjection procedure.

After the 24 h water deprivation period animals were presented with water for 15 min per day from a graded bottle during 4 days to establish baseline water consumption. On the fifth day, animals were assigned to different groups by taking into consideration the average baseline water consumption. The neophobic response was tested by the presentation of 0.5% saccharin (Sigma, St. Louis, MO) for 15 min (first presentation of saccharin), followed by access to water for 15 min, to ensure that all animals consumed their daily fluid requirement regardless of their saccharin consumption. The day after, all the animals had access to the saccharin solution for 15 min (second presentation of saccharin, AN). The neophobic response and AN are expressed as percentage of baseline consumption = 100 × (saccharin solution intake/mean baseline water consumption).

2.5. Histology

After completion of experiments, animals were overdosed with sodium pentobarbital, and intracardially perfused with 0.9% saline solution followed by buffered 4% paraformaldehyde. The brains were removed and stored at 4–10 °C in the same paraformaldehyde solution during 24 h, and then removed and put in a 30% glucose solution until tissue saturation was reached. Brains were cut on a freezing cryostat in 40 μm coronal slices. Mounted samples were stained with cresyl violet and examined under light microscope to determine cannulae placement.

3. Results

The injection sites cover the three layers that comprise the IC (granular, disgranular and agranular layers) (see Fig. 1). Eleven animals with cannulae misplacements were discarded.