Behavioural Brain Research 278 (2015) 202–209

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Chemical stimulation or glutamate injections in the nucleus of solitary tract enhance conditioned taste aversion

Nadia Edith García-Medina, Gabriela Vera, María Isabel Miranda*

Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Boulevard Juriquilla No. 3001, Juriquilla, Querétaro, Qro. 76230, Mexico

HIGHLIGHTS

- Glutamate in the amygdala correlates with the strength of taste aversive memory.
- Nucleus of the solitary tract stimulation sustains amygdala glutamate levels.
- Glutamate in the nucleus of the solitary tract induces strong taste aversion learning.
- Norepinephrine release in amygdala is induced by the solitary tract depolarization.

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 2 September 2014
Accepted 15 September 2014
Available online 22 September 2014

Keywords:
Taste learning
Glutamate
Solitary nucleus tract
Basolateral amygdala
Aversive stimulus
Aversive memory

ABSTRACT

Taste memory depends on motivational and post-ingestional consequences after a single taste–illness pairing. During conditioned taste aversion (CTA), the taste and visceral pathways reach the nucleus of the solitary tract (NTS), which is the first relay in the CNS and has a vital function in receiving vagal chemical stimuli and humoral signals from the area postrema that receives peripheral inputs also via vagal afferent fibers. The specific aim of the present set of experiments was to determine if the NTS is involved in the noradrenergic and glutamatergic activation of the basolateral amygdala (BLA) during CTA. Using in vivo microdialysis, we examined whether chemical NTS stimulation induces norepinephrine (NE) and/or glutamate changes in the BLA during visceral stimulation with intraperitoneal (i.p.) injections of low (0.08 M) and high (0.3 M) concentrations of lithium chloride (LiCl) during CTA training. The results showed that strength of CTA can be elicited by chemical NTS stimulation (Ringer's high potassium solution; 110 mM KCl) and by intra-NTS microinjections of glutamate, immediately after, but not before, low LiCl i.p. injections that only induce a week aversive memory. However visceral stimulation (with low or high i.p. LiCl) did not induce significantly more NE release in the amygdala compared with the NE increment induced by NTS potassium depolarization. In contrast, high i.p. concentrations of LiCl and chemical NTS stimulation induced a modest glutamate sustained release, that it is not observed with low LiCl i.p. injections. These results indicate that the NTS mainly mediates the visceral stimulus processing by sustained releasing glutamate in the BLA, but not by directly modulating NE release in the BLA during CTA acquisition, providing new evidence that the NTS has an important function in the transmission of signals from the periphery to brain systems that process aversive memory formation.

1. Introduction

Taste memory formation begins when the branches of the facial, glossopharyngeal, and vagus nerves are activated and transmit taste activation to the rostral part of the nucleus of the solitary tract (NTS) [1], which is the first central synaptic relay for gustatory information [2] and has a vital function in receiving vagal chemical stimuli and humoral signals from the area postrema that receives peripheral inputs also via vagal afferent fibers [48–50]. The NTS has an important role in modulating sensory aspects of gustatory function; for example, electrolytic lesions of the rostral NTS impair the response to sapid stimuli, as well as the innate gustatory preference or aversion; however, animals with lesions in the NTS can still use taste cues for learning aversion [3]. During taste memory formation, taste and visceral pathways converge in several brain structures such as the NTS, parabrachial nucleus (PBN), thalamus, central and

* Corresponding author. Tel.: +52 442 2381039; fax: +52 55 56234046.
E-mail address: mirandami@unam.mx (M.I. Miranda).

http://dx.doi.org/10.1016/j.bbr.2014.09.023
0166-4328/

basolateral nucleus of the amygdala (CEA and BLA, respectively), and insular cortex, where taste and malaise can be associated to create the taste memory representation [4]. The NTS in particular receives projections from the CEA and BLA [5]; electrophysiological studies demonstrated that the firing interval in the amygdala neurons increases significantly after electrical stimulation of NTS neurons [6,7], and vagal nerve stimulation produces a significant increase in the number of amygdala neurons that express *Fos* [8]. Consequently, recent studies demonstrate an important NTS-BLA interaction during memory formation [9–11].

NTS neurons also project directly to the locus coeruleus [12,13], the structure that provides the major source of norepinephrine (NE) innervations to the BLA as well as to several other structures [14,15]. Thus, vagal activation of the NTS may initiate NE release in the amygdala, either by direct NTS action on the BLA or via a polysynaptic pathway involving the locus coeruleus [11,16]. There is evidence that β-adrenoreceptors in the BLA are involved during taste or taste-malaise association, since microinfusions of the β-adrenergic antagonist propranolol into the BLA and insular cortex prevented incidental taste learning but did not affect associative/aversive taste learning [17].

In addition to these findings, the amino acid glutamate is another neurotransmitter involved during synaptic communication between vagal afferents and neurons in the NTS [18]. Vagal afferents contain glutamate [19], which is significantly increased in the NTS by direct stimulation of vagal afferents [20]. During conditioned taste aversion (CTA), a visceral stimulus (e.g., LiCl injection) induces a dramatic increase in glutamate release in the amygdala, indicating that glutamatergic activation of the amygdala can partially imitate the visceral stimulus during CTA [16,21]. The current evidence highlights the importance of the NTS for understanding the mechanism of vagal-mediated memory enhancement, since the NTS is the target area for vagal afferents, as well as inputs from area postrema, and contains neurons that project to regions of the brain such as the amygdala that exhibit activity after periphery stimulation [6,22–25]. Although the ability of vagal or NTS input to influence the amygdala has been demonstrated in previous research, there is still scarce information about the neurotransmitters, and the degree to which their transmission is mediated by the NTS during the convergence of taste and visceral stimuli that allows taste memory formation.

Accordingly, the main goal of this research was to evaluate whether the activity of the NTS promotes NE and glutamate release in the BLA upon weak visceral stimulation during the acquisition of CTA. First, we examined whether chemical NTS stimulation (Ringer's high potassium solution; 110 mM, KCl) or intra-NTS microinjections of glutamate, immediately before or after low (0.08 M) LiCl i.p. injections are able to induce taste aversive learning when saccharin is paired with low visceral stimulation. Second, using in vivo microdialysis, we examined whether chemical NTS stimulation or intra-NTS glutamate injections, induce NE and/or glutamate changes in the BLA during CTA training, and if these stimulation changes are correlated with CTA strength.

2. Materials and methods

2.1. Animals

A total of 119 Sprague–Dawley male rats were used for pharmacology and microdialysis experiments (Instituto de Neurobiología breeding colony, weighing 250–300 g). Animals were individually housed with access to food and water (except during behavioral tests) and maintained at 23 °C in an inverted 12-h/12-h dark–light cycle. All experimental procedures were performed during the dark phase, given that rats have a nocturnal activity pattern. Experiments were performed in accord with the rules in health matters (Ministry of Health, Mexico), according to the Mexican Laws for Animal Care (Norma Oficial Mexicana SAGARPA) and with the approval of the local Animal Care Committee (Comité de Bioética del Instituto de Neurobiología de la UNAM), complied with the NIH Guide for Care and Use of Laboratory Animals and Rules in Health Matters (Ministry of Health, México).

2.2. Guide cannula implantation

All animals were anaesthetized with ketamine (70 mg/kg) and xylazine (6 mg/kg) i.p. First, to evaluate if activation of the NTS promotes CTA, rats were implanted with bilateral, 23-gauge stainless steel cannulae 2.0 mm above the NTS (AP = −13.3 mm, *L* = ±1.0 mm, *V* = −5.6 mm from bregma). Second, to evaluate by microdialysis the effects of stimulation on the release of NE or glutamate during CTA, animals were implanted with bilateral NTS stainless steel cannulae and a unilateral 2.0-mm microdialysis guide (BAS, West Lafayette, IN) above the left BLA (AP = −2.8, *L* = ±5.0 mm, *V* = −6.5 mm from bregma) [26]. Cannulae were fixed to the skull with two surgical screws and dental acrylic cement. Stylets were inserted into the guide cannulae to prevent clogging.

2.3. Conditioned taste aversion

Five days after surgery, when complete recovery was already observed, rats were water deprived for 18 h and then were daily habituated to drink water, from a spout inserted into a graduated cylinder, for 15 min per day for 5 days until a stable water consumption baseline was reached. On day 6, CTA acquisition underwent as previously reported [21,27]. Briefly, a novel taste, 0.1% saccharin solution, was presented to the rats for 15 min, and 30 min after the end of the drinking period they were i.p. injected with LiCl (0.3 M, 10 ml/kg) to induce gastric malaise (or with 0.08 M LiCl, which does not induce a significant aversive conditioning). The next day (24 h later), the CTA memory retrieval test was carried after presenting 0.1% saccharin solution only. Taste aversion was evaluated as the decrease in % CTA acquisition: ml consumption during retrieval × 100/ml consumption during acquisition. A strong aversive memory formation was considered when a reduction in saccharin consumption during retrieval day was equal or less than 50% of the consumption observed during acquisition day.

2.4. Experiment 1: Effects of chemical or glutamatergic NTS stimulation before or after visceral stimulation during CTA acquisition

First we evaluated whether the activation of the NTS promotes the acquisition of CTA. To do this, we followed previous evidence demonstrating that animals injected i.p. with a low dose of LiCl (low, 0.075 M) are not able to acquire a significant taste aversion, compared with animals that received a higher dose of LiCl (Control, 0.3 M or 0.4 M), which induces a clear CTA [21,28]. Accordingly, using this experimental approach, we tested if NTS chemical activation, with high potassium Ringer's solution infused before low LiCl i.p injections, was able to enhance visceral processing and induce CTA. The infusions were made immediately before LiCl, to distinguish the time convergence between unconditioned stimulus (US) and the effects of high potassium, on neurotransmitter release in the BLA, during CTA acquisition. Thus, on CTA acquisition day, animals were randomly separated in the following groups: (1) Control CTA group animals (CONTROL), were infused in the NTS with Ringer's solution (82 mM KCl) immediately before i.p. injections of