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Research article

Local infusion of ghrelin into the lateral amygdala blocks extinction of conditioned taste aversion in rats

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ABSTRACT

Ghrelin is an orexigenic brain-gut hormone promoting feeding and regulating energy metabolism in human and rodents. Our previous study showed that ghrelin locally infused into the lateral amygdala (LA) activates its receptor GHS-R1a and blocks acquisition of conditioned taste aversion (CTA) in rats. In this study, we further investigated the effect of ghrelin/GHS-R1a signaling on extinction of CTA. We found that local infusion of ghrelin (5 μM, 0.5 μl/side) into the LA not only interfered with CTA memory formation, but also the extinction of CTA memory. Pre-administration of GHS-R1a antagonist blocked ghrelin's effect on both CTA acquisition and extinction. However, pre-treatment with PI3K inhibitor only abolished the inhibitory effect of ghrelin on acquisition, but not on extinction. Altogether, our data indicated that ghrelin/GHS-R1a signaling in the LA circuit modulates both acquisition and extinction of CTA, the two forms of taste aversion processes with distinct mechanisms may also share certain molecular and circuit components in common.

1. Introduction

Ghrelin is an acylated, neuropeptide hormone promoting food intake and regulating energy metabolism in human and rodents [1]. Endogenous ghrelin receptor, the growth hormone secretagogue receptor 1a (GHS-R1a), are widely expressed in both hypothalamus and extra-hypothalamic regions [2]. An increasing number of studies have reported that beyond feeding control and energy metabolism, ghrelin/GHS-R1a signaling plays complex roles in modulating advanced activities of the central nervous system, including mood, learning, memory and etc. [3].

Conditioned taste aversion (CTA) is an established learning paradigm used to study the molecular, cellular, circuit and systemic mechanisms of acquisition and extinction of non-declarative memory. In CTA, the subject learns to associate a novel taste (conditioned stimulus, CS) with a subsequent visceral illness (unconditioned stimulus, US). A number of anatomical and pharmacological studies have shown that the neural network involved in CTA acquisition and retention includes the nucleus of the solitary tract, the parabrachial nucleus, the medial thalamus, the amygdala, and the insular cortex [4]. CTA extinction is defined as a form of learning in which previous associations between US and CS are weakened by repeated memory retrieval in the absence of the US. Previous studies showed that the neural circuit involved in CTA memory extinction should include insular cortex, the amygdala, ventromedial prefrontal cortex, the parabrachial nucleus, and the nucleus of the solitary tract [5–8]. Apparently, most of the neuronal circuits that sub-serve CTA acquisition also participate in extinction, among which the amygdala circuits is of particular interest.

The amygdala is one of the key structures essential for acquisition and storage of multiple types of aversive and emotional memory, including auditory fear conditioning and CTA [9–11]. In particular, the nuclei of lateral amygdala (LA) receives multimodal sensory input from the thalamus and cortex, and is considered to serve as an essential site where NMDA receptor-dependent synaptic plasticity is required for associative learning and memory formation. Our previous study showed that ghrelin locally infused into the lateral amygdala (LA) blocks acquisition, but not consolidation, of CTA [12]. Since the amygdala is involved in both acquisition and extinction of the CTA memory, in this study we micro-infused ghrelin into the LA and further investigated the possible effect of ghrelin/GHS-R1a signaling on CTA extinction. Moreover, since previous study showed that local infusion of ghrelin regulates hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase (PI3K) signaling in the dentate gyrus of adult rats [13], we were interested to know whether PI3K activation in the LA circuit contributes to ghrelin's effect on CTA acquisition and extinction.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (300–350 g) used in the experiments were

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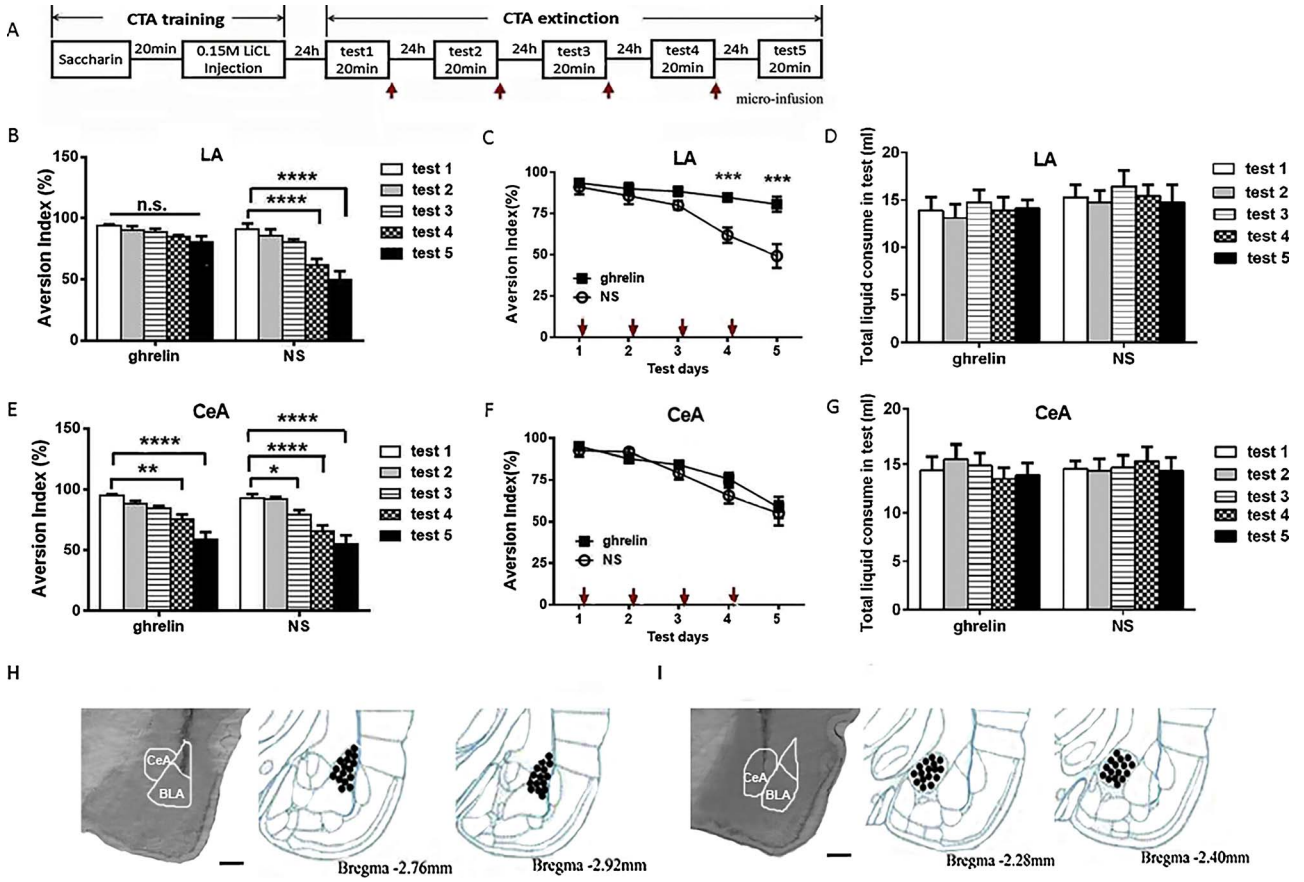

Fig. 1. Intra-LA infusion of ghrelin blocks CTA extinction. (A) Illustration of the experimental design. Rats receive bilateral micro-infusion of ghrelin or vehicle into the LA (B–D, H) or the CeA (E–G, I) immediately after CTA tests which were performed for 5 consecutive days. Arrow indicates the time of drug infusion. (B, E) Aversion performance. B, intra-LA infusion. NS-treated rats exhibit extinction while ghrelin-treated rats did not, n = 7–8 each group. E, intra-CeA infusion. Both groups show gradually declined AI. n = 8–9 per group. (C, F) Extinction learning curve. C, intra-LA infusion. F, intra-CeA infusion. (D, G) Total liquid consumption during CTA tests. D, intra-LA infusion. G, intra-CeA infusion. (H–I) Representative photomicrographs of microinjections into the LA (H) and the CeA (I) and outline of the injection sites on cross-sections from Paxinos and Watson (2007). Scale bars represent 0.5 mm. All data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 or ****p < 0.0001 means significant difference, n.s. indicates not significant.

purchased from the Experimental Animal Center at Lukang Pharmaceutical Co (Jining, China). Rats were single-housed at controlled temperature (20–22 °C) and under 12 h light/12 h dark cycle. All behavioral procedures were conducted during the light phase of the cycle. All procedures were performed in accordance with the National Institutes of Health *Guide for the care and use of laboratory animals* and were approved by the Chancellor's Animal Research Committee of Qingdao University.

2.2. Surgical procedures and micro-infusion

Rats were anesthetized with 8% chloral hydrate (400 mg/kg, i.p) and 23-gauge stainless steel guide cannulas were implanted bilaterally into either the LA or the central nucleus of the amygdala (CeA) with the guidance of a stereotaxic apparatus (RWD Life Science). The stereotactic coordinates of LA were anteroposterior −2.65 mm, mediolateral ± 5.2 mm and dorsovenral −7.5 mm relative to bregma; the CeA coordinates were anteroposterior −2.2 mm, mediolateral ± 4.0 mm and dorsoventral −7.0 mm relative to bregma, according to Paxinos and Watson (1998). The cannulas were anchored to the skull with acrylic dental cement and secured with skull screws. Animals were allowed to recover from surgery for 7 days before experimental manipulations. For micro-infusion, a 28-gauge infusion cannula, extending 0.8 mm beyond the tip of the guide cannula, was inserted. The infusion cannula was connected via PE20 tubing to a Hamilton micro-syringe driven by a micro-infusion pump (Stoelting Co., USA). Micro-infusion was performed bilaterally (0.5 µl/side) with an infusion speed of 0.1 µl/min.

Chemicals or vehicles (pH 7.4) were locally infused into either the LA or the CeA with equal volume (0.5 µl/side). Rat acyl-ghrelin (5 µM, dissolved in normal physiological saline), GHS-R1a antagonist YIL718 (750 µM, in saline) and PI3K inhibitor LY294002 (6 µM, in saline containing 0.15% DMSO) were purchased from R & D Systems (Minneapolis, MN, USA). Lithium chloride (LiCl) and saccharin were purchased from Sigma (St Louis, MO, USA). Drug dosages used for micro-infusion as well as the timing of drug administration were chosen according to previous reports or our preliminary results [12,14,15].

2.3. Behavioral procedure

CTA training and extinction were performed according to the previous study [7] with modification. Briefly, saccharin (0.1% w/v) was used as CS and intra-peritoneal injection of LiCl (0.15 M, 2% body weight) as US. Rats were deprived of water for 24 h, and then habituated over 5 days to obtain their daily water supply within 20 min from two serological pipettes each containing 10 ml of water. On training day, rats were presented with 0.1% saccharin instead of water for 10 min. Twenty minutes later, they were injected intraperitoneally with 0.15 M LiCl solution. Twenty-four hours after conditioning, a multiple-choice test was performed to evaluate the acquired aversion to saccharin. Rats were presented with an array of 6 pipettes for 20 min, three containing 5 ml saccharin and three containing 5 ml water in a pseudo-random order. For extinction learning, the conditioned rats were presented once a day with the same multiple-choice situation for 5 consecutive days. The aversive memory to saccharin were quantified by