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Ghrelin Affects Certain Forms of Learning and Memory in both Rats and Mice

Yao Hui Wang[1]

[1]Department of Physiology, Medical College of Qingdao University, Qingdao, Shandong 266071, China

Abstract: Ghrelin is an recently discovered peptide hormones that play opposite roles in the food intake, body-weight control and energy homeostasis in both human and rodents. Beyond its appetite-control function, increasing evidence has shown that ghrelin affects multiple advanced activities in the central nervous system, including memory and emotion. However, the possible actions of ghrelin in those important brain regions are largely unknown. In this study, we micro-infused ghrelin into the lateral amygdala (LA) or area CA1 of the dorsal hippocampus (CA1) and investigated the immediate effects of those two peptide hormones on cognitive and affective behaviors. We found that the micro infusion of ghrelin into the LA or the CA1 interfered with certain types of learning and memory in both rats and mice. Our data thus suggested that ghrelin affects learning and memory.

Keywords: Ghrelin;GHS-R1a;Amygdala;Hippocampus;Memory

Introduction:

Ghrelin is an octanoylated, 28-amino acid orexigenic peptide which is the only identified endogenous ligand of growth hormone secretagogue receptor 1a (GHS-R1a) (Kojima et al., 1999; Sato et al., 2005). Ghrelin was synthesized peripherally in the stomach and centrally in the hypothalamus (Kojima et al., 1999; Sato et al., 2005; Goebel et al., 2009a). Particularly, it was recently reported that the majority of gastric X/A-like endo-crine cells producing nesfatin-1 also express and release ghrelin (Stengel et al., 2010). As peptide hormones, ghrelinand nesfatin-1 act inversely in the hypothalamus to control food intake and body weight. Ghrelin is well established to stimulate food intake in humans and various animal species by central and peripheral mechanisms (Howard et al., 1996; Kojima et al., 1999; Tschop et al., 2000; Mitchell et al., 2001).Besides in the hypothalamic pathways regulating food intake and energy homeostasis, ghrelin/GHS-R1a is also expressed in numerous extra-hypothalamic neuronal popu-lations, including cortex, hippocampus, amygdala and many others (Mitchell et al., 2001; Zigman et al., 2006; Ferrini et al., 2009; Goebel et al., 2009a; Cong et al., 2010; Goebel-Stengel et al., 2011; Palasz et al., 2012). The widespread, somehow overlapped distribution of these neuropeptide in the central nervous systems (CNS) suggests that it may play broader and related biological actions beyond the well-established feeding controls.impairments in spatial learn-ing (Davis et al., 2011) and exogenous ghrelin rescued deficits shown by ghrelin knockout (ghrelin-/-) mice in a novel object recognition test (Diano et al., 2006). In contrast to memory enhancement, memory impairments after ghrelin administra-tion has been reported in neonatal chicks (Carvajal et al., 2009). A very recent study further showed that GHS-R1a knockout mice exhibited clearly better performance in Morris water maze, suggesting that GHS-R1a activation actually interferes with acquisition of spatial memory (Albarran-Zeckler et al.,

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Yao Hui Wang (Correspondence)
wangyaohuiwyh@yeah.net

2012). In human studies, serum ghrelin levels were recently shown to be negatively correlated with declarative memory in elderly adults (Spitznagel et al., 2010). Also, ghrelin seemed to impair rather than promote procedural memory consolidation (Dresler et al., 2010). Despite all those discrepancies, it is clear that central ghrelin and GHS-R1a signaling regulates cognitive and affective behavior, although the precise mechanisms underlying those processes are not certain yet.

It is well known that both hippocampus and the amygdala play critical roles in control of the cognitive and affective behaviors. Hippocampus is a crucial structure for the process of certain types of memory such as the episodic and spatial memory, and emotional responses as well (Silva et al., 1998; Frankland et al., 2004; Zhou et al., 2013). Amygdala is one of the key brain structures implicated in the acquisition and storage of multiple types of aversive and emotional memory (LeDoux, 2000; McGaugh, 2002; Ehrlich et al., 2009; Zhou et al., 2009). Thus, to investigate the actions of ghrelin and nesfatin-1 in those important brain regions involved in emotion, learning and memory, we infused ghrelin or nesfatin-1 into the CA1 region of the mice hippocampus (CA1) and the lateral amygdala (LA) of rats. The effects of ghrelin and nesfatin-1 on cognitive and affective behaviors were then assessed in parallel with behavioral paradigms including Morris water maze (MWM), open field, elevated plus maze and conditioned taste aversion (CTA). The main reason for the use of both mice and rats instead of single animal species is to test whether the modulatory effects of ghrelin and nesfatin-1 on behaviors are universal to different animal species. The aim of this study is to discovery ghrelin on certain forms of learning and memory that depend on different neural networks.

Results

Intra-LA infusion of ghrelin blocked CTA in rats

In our CTA training paradigm, 150 mM LiCl solution was used to establish the aversive memory since drinking LiCl induces nausea response in rats, meanwhile its salty taste makes the animal acquiring avoidance to another similar salty solution, for example 50 mM NaCl. Our previous studies have demon-strated that NMDA receptor or AMPA receptor activation in the LA are required for the acquisition and expression of such type of aversive memory respectively (Song et al., 2013), indicating that the LA is an important structure for CTA memory processing. Thus, we infused ghrelin and nesfatin-1 into the LA before training and used this behavioral paradigm to compare their effect on the formation of the aversive memory to taste.

Consistent with our previous findings (Song et al., 2013), we showed here that micro infusion of ghrelin (12 ng, 0.5 μl per side) into the LA 20 min before training interfered with the acquisition of CTA memory. As shown in Fig. 1A, ghrelin treatment group displayed significant smaller AI (43.6710.5%, n¼8) compared to the vehicle group (75.9%76.0%, n¼8) as tested at 24 h after conditioning (unpaired t-test, po0.05). Importantly, the ghrelin-treated rats and vehicle-treated rats consumed similar volume of liquid during both training (Fig. 1B) and tests (Fig. 1C). To rule out the possibility that the blockade of ghrelin on CTA acquisition was due to physical damage induced by cannulation or toxic effect of ghrelin, we indeed, increasing evidence has shown that ghrelin affects multiple higher CNS activities including the emotion and mem-ory. For example, earlier studies reported that ghrelin adminis-tration induced anxiogenesis in both rats and mice (Asakawa et al., 2001; Carlini et al., 2002, 2004; Kanehisa et al., 2006). However, a recent study suggested that subcutaneous injec-tion of ghrelin produced anxiolytic- and antidepressant-like responses in mice (Lutter et al., 2008). Ghrelin or the ghrelin mimetic LY444711 was previously reported to produce a marked improvement in spatial memory recall in rats after subcutaneous injection (Diano et al., 2006). Intracerebroventricular (icv) admin-istration of ghrelin improved retention of certain types of memory in mice (Diano et al., 2006). Consistently, ghrelin receptor deficient mice expressed re-trained the same groups of rats on CTA to another unfamiliar taste, glycine (1%

w/v), 24 h after com-pleting the above experiments. In the new CTA to glycine, i.p. injection of 0.15 M LiCl (2% body weight) induced malaise. Obviously, two groups of rats acquired identical CTA to glycine, the new conditioned stimulus (Fig. 1D), indicating that the basolateral complex of amygdala was not damaged or silenced by cannulation or ghrelin. Therefore, the effect of ghrelin on CTA acquisition is possibly due to the specific interruption of CS-US association in the LA by certain mechanisms.

Next, we checked the possible effect of nesfatin-1 on CTA acquisition with the same behavioral paradigm. Twenty minutes before training, experimental rats received bilateral infusion of either 2.4 ng, 48 ng or 240 ng nesfatin-1 into the LA, respectively. The control animals received the same volume of saline injection (0.5 µl per side). The drug doses used was chosen based on previous reports showing that icv injection of nesfatin-1 (48 ng or 240 ng) suppressed food intake (Oh et al., 2006) and increased anxiety in the rat (Merali et al., 2008).

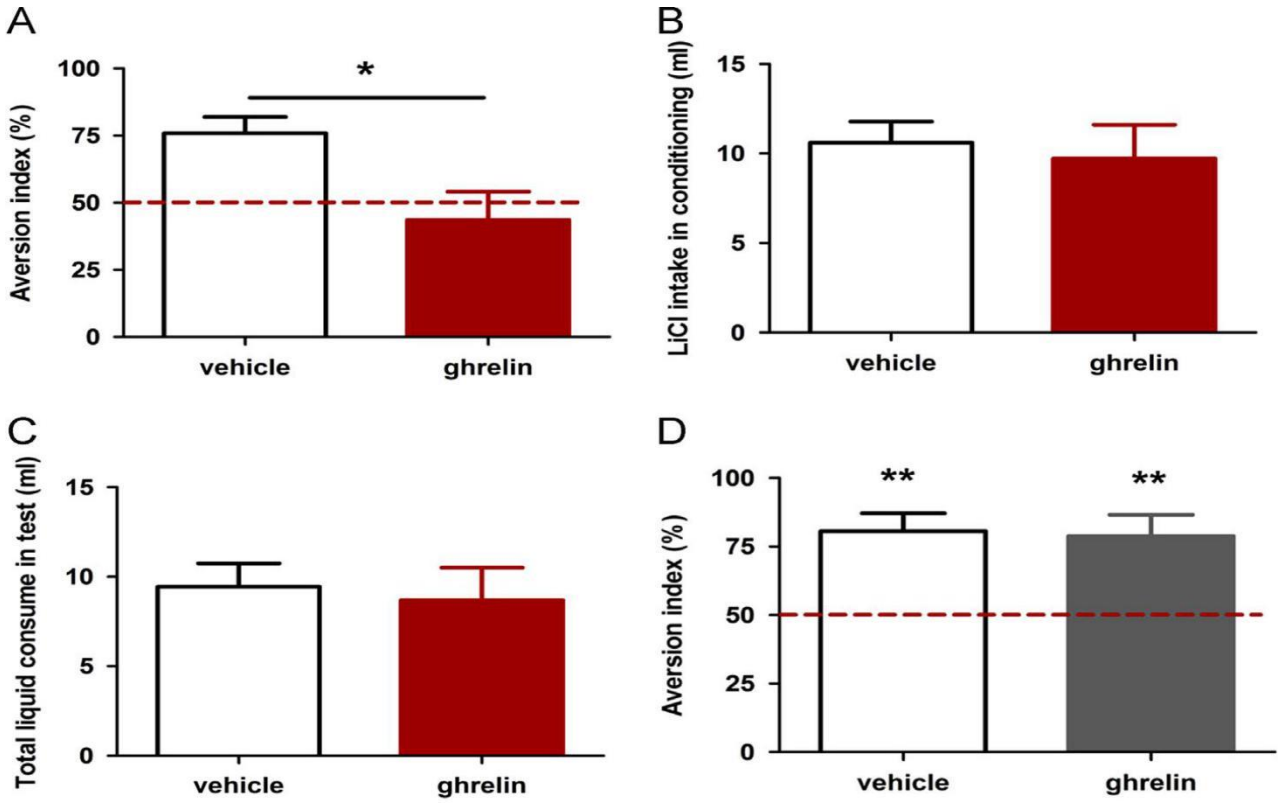

Fig. 1– Micro infusion of ghrelin into CA1 of the dorsal hippocampus impairs spatial learning and memory in mice, while nesfatin-1 does not. (A) Time to find the hidden platform plotted versus training blocks (2 blocks for each training day) in ghrelin-treated (12 ng, 0.5 µl) and control animals. (B) Platform crossing times during the probe test conducted 24 h after completion of training, showing that ghrelin-treated mice had spatial memory deficits. Two-way repeated measure ANOVA, location x treatment interaction F(3, 51) ¼5.5, po0.01, Bonferroni post test, nnnpo0.001 means significant difference between quadrants, nnpo0.01 means significant difference between groups. n¼9–10 for each group. (C) Percentage time spent in each quadrant during probe trial. Time spent in target quadrant (TQ), adjacent left quadrant (AL), opposite quadrant (OP) and adjacent right quadrant (AR) are labeled as indicated. Mice received nesfatin-1 (240 ng, 0.5 µl) or vehicle infusion showed strong spatial bias for the target quadrant where the platform was located during training. Two-way repeated measure ANOVA, significant quadrant variation F(3, 36)

¼16.3, po0.0001, Bonferroni post test, npo0.05 or nnpo0.01 means significant difference compared to other quadrants. n¼7 for each group. (D) The number of platform crossing was also same for two groups. Two-way repeated measure ANOVA, significant quadrant F(3, 42) ¼19.9, po0.0001, Bonferroni post test, nnpo0.01 or nnnpo0.001 means significant difference compared to other locations. n¼7 for each group. All data are shown as mean7SEM.

Discussion

Many of the feeding hormones, for example insulin, leptin, ghrelin, etc, have effects on learning, memory, attention, and other aspects of cognition (Diano et al., 2006; Paz-Filho et al., 2008; Stranahan et al., 2008), probably due to the fact that feeding requires certain cognitive aspects like decision mak-ing. Ghrelin is recently discovered peptide hormones that can cross the blood–brain barrier (BBB) to exert their CNS effects. Interestingly, ghrelin play opposite roles in food intake, body-weight control and energy homeostasis in both human and rodents. Therefore, we were interested to know whether these two peptide hormones played correlative roles in modulating memory and emotion. With intra-nucleus micro infusion and a battery of behavioral analyses, we examined the effect of ghrelin on cognition and emotion in both rats and mice. Our results showed that intra-CA1 (intra-LA) infusion of ghrelin blocks the acquisition of hippocampus-dependent spatial memory (amygdala-dependent aversive memory).

Consistent with our previous findings (Song et al., 2013), we provided more evidence here that ghrelin blocks memory formation. Ghrelin's blockage on memory processes seems to be a general phenomenon since it acts in different brain regions and different animal species. Although many previous studies have shown that ghrelin enhances memory processes (Carlini et al., 2002, 2004; Diano et al., 2006; Davis et al., 2011), memory impairments induced by ghrelin admin-istration have been reported recently in chicken, rodent and human studies (Carvajal et al., 2009; Dresler et al., 2010; Spitznagel et al., 2010; Albarran-Zeckler et al., 2012). The experimental procedure, the sensitivity of the behavioral paradigms, the genetic background and the age of animals may account for some of the discrepancies among different studies. Noticeably, we administrated a smaller amount of ghrelin compared to others (Carlini et al., 2004; Toth et al., 2008; Alvarez-Crespo et al., 2012; Goshadrou and Ronaghi, 2012). In addition, we infused ghrelin into the specific brain regions (LA and CA1) instead of ip or icv injection used by previous studies (Carlini et al., 2002; Diano et al., 2006; Carlini et al., 2008; Hansson et al., 2011).

Not only affected learning and memory, previous studies demonstrated that ghrelin caused complicated, even contro-versial effects on emotional responses including anxiety and depression (Asakawa et al., 2001; Carlini et al., 2002, 2004; Kanehisa et al., 2006; Lutter et al., 2008), which could have big.

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