Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Ghrelin but not nesfatin-1 affects certain forms of learning and memory in both rats and mice

Qianqian Zhu[a,1], Kewei Xiao[a,1], Ming Yu[a], Minglu Niu[a], Chengqian Li[b], Yanxia Gao[b], Guo-Dong Li[c,*], Yu Zhou[a,**]

[a]Department of Physiology, Medical College of Qingdao University, Qingdao, Shandong 266071, China
[b]Department of Internal Medicine, Medical College Affiliated General Hospital, Qingdao, Shandong 266021, China
[c]Department of Anesthesiology, Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Accepted 13 October 2013
Available online 19 October 2013

Keywords:
Ghrelin
GHS-R1a
Nesfatin-1
Amygdala
Hippocampus
Memory

ABSTRACT

Ghrelin and nesfatin-1 are two 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. Nesfatin-1 was also widely expressed in extra-hypothalamic brain regions including hippocampus and amygdala. However, the possible actions of nesfatin-1 in those important brain regions are largely unknown. In this study, we micro-infused ghrelin or nesfatin-1 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, while nesfatin-1 had no effect. Our data thus suggested that although nesfatin-1 works as a functional antagonist of ghrelin in the feeding control, only ghrelin affects learning and memory.

1. 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). Nesfatin-1 is a newly discovered, 82-amino acid anorexigenic peptide which is derived from a precursor protein, nucleobindin-2 (Oh et al., 2006). Both ghrelin and nesfatin-1 were 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 endocrine cells producing nesfatin-1 also express and release ghrelin (Stengel et al., 2010). As peptide hormones, ghrelin

*Correspondence to: Department of Anesthesiology, David Geffen School of Medicine, University of California at Los Angeles, 757 Westwood Plaza, Suit 3304, Los Angeles, CA 90095, USA.
**Correspondence to: Department of Physiology, Medical College of Qingdao University, 308 Ningxia Road, Room 403B, Boya Building, Qingdao, Shandong 266071, China.
E-mail addresses: guodongli@mednet.ucla.edu (G.-D. Li), yuzhou7310@gmail.com (Y. Zhou).
[1]Authors made equal contributions.

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.10.022

and 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). Nesfatin-1, however, is a potent satiety factor and strongly inhibits food and water intake after central or peripheral administration (Shimizu et al., 2009; Stengel et al., 2010; Stengel and Tache, 2010; Palasz et al., 2012). Besides in the hypothalamic pathways regulating food intake and energy homeostasis, ghrelin/GHS-R1a and nesfatin-1 are also expressed in numerous extra-hypothalamic neuronal populations, 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 two neuropeptides in the central nervous systems (CNS) suggests that they may play broader and related biological actions beyond the well-established feeding controls.

Indeed, increasing evidence has shown that ghrelin affects multiple higher CNS activities including the emotion and memory. For example, earlier studies reported that ghrelin administration 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 injection 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) administration of ghrelin improved retention of certain types of memory in mice (Diano et al., 2006). Consistently, ghrelin receptor deficient mice expressed impairments in spatial learning (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 administration 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., 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.

Similar to ghrelin, recent findings suggest that, far beyond being only a hypothalamic peptide with potent anorexigenic actions, nesfatin-1 induces a wide spectrum of central activities, one of which is the stress and associated emotional responses, such as anxiety and depression. Various acute stressors can activate the nesfatin-1 expression in stress-responsive neuronal populations in the brain (Bonnet et al., 2009; Goebel et al., 2009b; Stengel et al., 2010; Goebel-Stengel et al., 2011), indicating its significance in the stress adaptation response (Yoshida et al., 2010; Yosten and Samson, 2010; Tanida and Mori, 2011). Meanwhile, studies showed that nesfatin-1 induced anxiety or fear (Oh et al., 2006; Merali et al., 2008). A higher serum nesfatin-1 level has also been revealed in patients with major depressive disorder (Ari et al., 2011). Compared to those evidence demonstrating that nesfatin-1 participates in emotion, it is still unclear whether nesfatin-1 affects memory, although it is widely expressed in the forebrain nuclei involved in memory processes, for example hippocampus and amygdala (Goebel-Stengel et al., 2011; Palasz et al., 2012).

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 compare the general effects of nesfatin-1 and ghrelin on certain forms of learning and memory that depend on different neural networks.

2. Results

2.1. *Intra-LA infusion of ghrelin blocked CTA in rats, while nesfatin-1 had no effect*

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 demonstrated 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 (43.6±10.5%, $n=8$) compared to the vehicle group (75.9%±6.0%, $n=8$)