Brain Research 1671 (2017) 26–32

Contents lists available at ScienceDirect

Brain Research

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

Research report

Central glucagon like peptide-1 inhibits reflex swallowing elicited by the superior laryngeal nerve via caudal brainstem in the rat

Motoi Kobashi [a,*], Satoshi Mizutani [a], Masako Fujita [a], Yoshihiro Mitoh [a], Yuichi Shimatani [b], Ryuji Matsuo [a]

[a] Department of Oral Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8525, Japan
[b] Department of Medical Engineering, Faculty of Engineering, Tokyo City University, Tokyo 158-8557, Japan

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 30 June 2017
Accepted 4 July 2017
Available online 8 July 2017

Keywords:
GLP-1
Incretin
Swallowing
AP
NTS

ABSTRACT

The effects of glucagon like peptide-1 (GLP-1) on reflex swallowing were examined using anaesthetized rats. GLP-1 was injected into the dorsal vagal complex (DVC) using glass micropipettes. Swallowing was induced by repeated electrical stimulation of the central cut end of the superior laryngeal nerve (SLN) and was identified by the electromyogram lead penetrated in the mylohyoide muscle through bipolar electrodes. Microinjection of GLP-1 into the medial DVC (M-DVC) increased the frequency of swallowing during the electrical stimulation of the SLN and extended the latency of the first swallowing. Microinjection of GLP-1 into the lateral DVC (L-DVC) did not change the frequency of swallowing or the latency of the first swallowing. Neither the injection of vehicle into the M-DVC nor L-DVC affected swallowing frequency. Pre-injection of exendin (5-39), a GLP-1 receptor antagonist, attenuated the degree of suppression of swallowing frequency induced by the administration of GLP-1 in addition to shortening the latency of the first swallowing. To identify the effective site of GLP-1, lesion experiments were performed. Electrical lesion of the commissural part of the NTS (cNTS) and the vacuum removal of the area postrema (AP) did not affect the inhibition of reflex swallowing induced by the injection of GLP-1 into the M-DVC. Electrical lesion of the medial nucleus of the NTS (mNTS) and its vicinity abolished the inhibitory effects of swallowing induced by the injection of GLP-1. These results suggest that GLP-1 inhibits reflex swallowing via the mNTS in the dorsal medulla.

1. Introduction

Swallowing is an early step in feeding behavior to propel food into the stomach, and is caused at the time between mastication and gastric accommodation. Both mastication and gastric accommodation are modified by orexigenic substances (Kobashi et al., 2002, 2009; Tsuji et al., 2011). It is therefore easily inferred that orexigenic and/or anorectic substances modify swallowing as well as other digestive functions. Our previous study revealed that the appetite-enhancing peptides, such as ghrelin and orexin, inhibit reflex swallowing induced by afferent stimulation of the SLN (Kobashi et al., 2010, 2014). Previous studies also demonstrated that the injection of a satiated peptide leptin into the rat dorsal medulla inhibited reflex swallowing induced by electrical stimulation of the SLN, as did ghrelin (Felix et al., 2006). Mostafeezur et al. revealed that cannabinoids, which have appetite-enhancing effects, shorten the latency of reflex swallowing (Mostafeezur et al., 2012). Thus, substances that modify food intake may affect reflex swallowing.

GLP-1 is an incretin hormone that enhances glucose-stimulated insulin secretion from pancreatic beta cells (Holst and Gromada, 2004; Vilsboll and Holst, 2004). GLP-1 is secreted from the L-cells of the gastrointestinal tract in response to food intake (Orskov et al., 1996 b). Other than hypoglycemic effects, GLP-1 reduces food intake (Tang-Christensen et al., 1996; Turton et al., 1996) and affects feeding related phenomena such as gastrointestinal motility and gastric acid secretion (Schirra et al., 2006; Wettergren et al., 1994, 1997).

The DVC, which involves the NTS, the DMV, and the AP, is situated in the middle of the caudal part of the dorsal medulla. The DVC is related to ingestive behavior and several autonomic

Abbreviations: AP, area postrema; DMV, dorsal motor nucleus of the vagus; DSG, dorsal swallowing group; DVC, dorsal vagal complex; L-DVC, lateral part of the DVC; M-DVC, medial part of the DVC; GLP-1, glucagon like peptide-1; NTS, nucleus tractus solitarius; cNTS, commissural part of the NTS; mNTS, medial nucleus of the NTS; SLN, superior laryngeal nerve.

* Corresponding author at: Department of Oral Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Shikata-cho 2-5-1, Kita-ku, Okayama 700-8525, Japan.
E-mail address: mkobashi@md.okayama-u.ac.jp (M. Kobashi).

http://dx.doi.org/10.1016/j.brainres.2017.07.004
0006-8993/

functions, and has dense projections from the periphery and higher center (Saper, 2004), including visceral sensory information, such as gastrointestinal, cardiovascular, and respiratory afferents.

The afferent fibers in the SLN respond to mechanical and chemical stimulation of the larynx and epiglottis (Bradley, 2000; Storey and Johnson, 1975). The SLN conveys sensory information to the L-DVC, which includes the NTS around the solitary tract and its vicinity (Furusawa et al., 1996). This region contains the swallowing pattern generator neurons, namely the DSG. The DSG receives both supramedullary inputs and peripheral afferents, such as the SLN, to elicit swallowing (Jean, 2001); therefore, the DVC, which includes the DSG, is the possible site of the feeding-related substances to regulate reflex swallowing. Indeed, ghrelin and leptin affect the vicinity of the solitary tract where the DSG is housed (Felix et al., 2006; Kobashi et al., 2010). On the other hand, orexin-A affects the M-DVC, which includes the AP, subpostrema region, the cNTS, the mNTS, and its vicinity (Kobashi et al., 2014). There may be regional specificity in the site of action of feeding-related peptides.

In the present study, we first confirmed how GLP-1 affects reflex swallowing elicited by electrical stimulation of the SLN using anaesthetized rats. We next determined the site of action of GLP-1 responsible for modifying reflex swallowing in the DVC.

2. Results

2.1. Suppressive effects of GLP-1 on reflex swallowing

The effects of microinjection of GLP-1 into the dorsal medulla on reflex swallowing elicited by repeated electrical stimulation of the SLN were examined. The drugs were injected into either the M-DVC or the L-DVC ipsilateral to the electrically stimulated SLN.

The injection of GLP-1 (20 nmol, 60 nl) into the M-DVC but not L-DVC decreased mean swallowing frequency and latency of the first swallow. The typical responses of EMG activities elicited by electrical stimulation of the SLN are shown in Fig. 1 A. Thirteen muscle contractions associated with swallowing occurred during electrical stimulation before the injection of GLP-1 (left panel). Ten minutes after the injection of GLP-1 into the M-DVC, the swallowing frequency fell (middle panel). Fifty minutes after the injection of GLP-1, the swallowing frequency returned to the level before administration (right panel). Fig. 1C shows the time course of the response before and after injecting the drugs. The time course of mean swallowing frequency (left) and mean latency (right) during electrical stimulation of the SLN are shown. The injection of GLP-1 into the L-DVC did not change the frequency of swallowing or the latency of the first swallowing (Fig. 1C).

The most reliable change was obtained ten minutes after the injection of GLP-1 (Fig. 1C). As shown in Fig. 2, the M-DVC is the effective site for GLP-1 (Fig. 2). Mean change in swallowing frequency after the injection of GLP-1 into the M-DVC was larger than that into the L-DVC ($F(1,24) = 6.270$, $P < 0.05$), and also larger than vehicle-injection into the M-DVC ($F(1,24) = 7.883$, $P < 0.01$). A significant interaction was observed ($F(1,24) = 5.533$, $P < 0.05$). The mean change in latency also demonstrated that the M-DVC is the effective site for GLP-1. The mean change in latency after the injection of GLP-1 into the M-DVC was longer than that into the L-DVC ($F(1,24) = 16.076$, $P < 0.001$), and also longer than vehicle-injection into the M-DVC ($F(1,24) = 23.456$, $P < 0.0001$). A significant interaction was observed ($F(1,24) = 18.325$, $P < 0.001$).

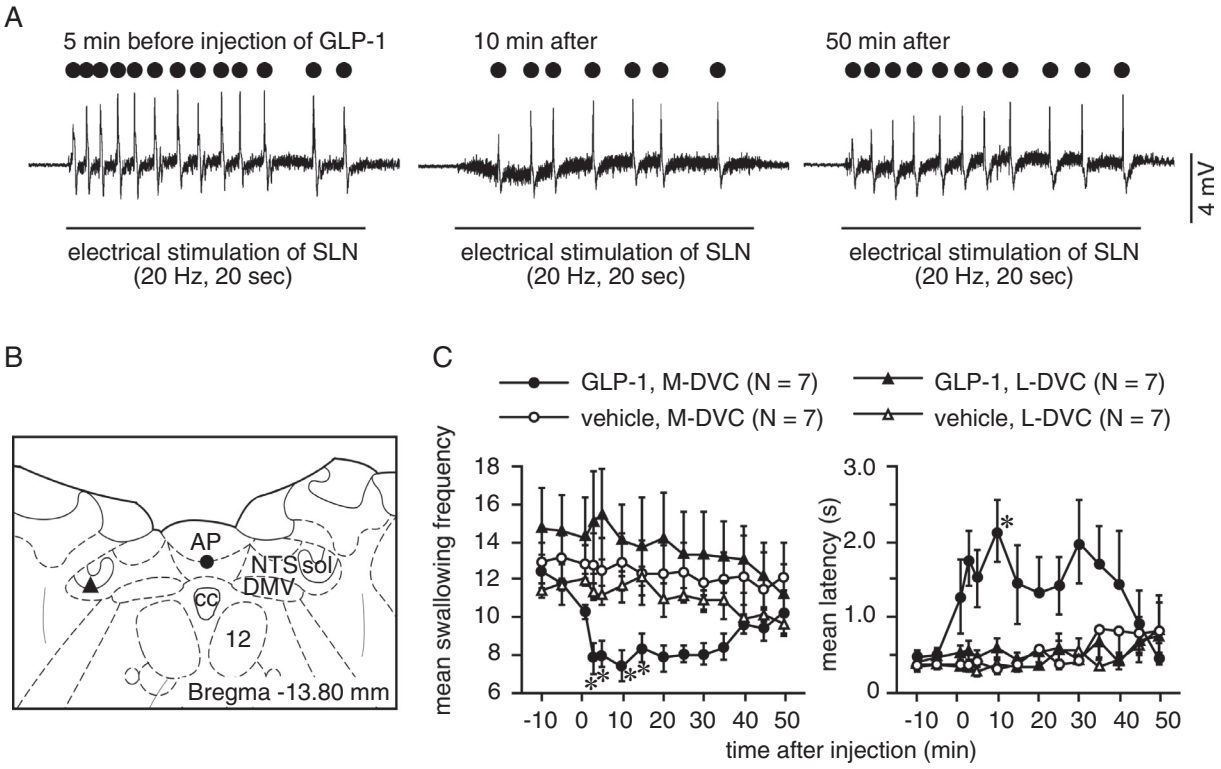

Fig. 1. Effects of injection of GLP-1 into the dorsal medulla on reflex swallowing induced by electrical stimulation of the SLN. A: EMG passing through bipolar electrodes penetrated the mylohyoid muscle. Repetitive swallowing was observed during electrical stimulation of the SLN for 20 s indicated by the horizontal bar. Filled circles indicate each swallowing movement. The injection of GLP-1 into the M-DVC (shown in B) inhibited reflex swallowing ("10 min after" in A). B: Schematic representation of the tip locations of the pipette for injection. The tip of the pipette was located in the lateral region of the NTS just below the solitary tract (L-DVC; filled triangle) or the boundary between the AP and the cNTS (filled circle). AP: area postrema, cc: central canal, DMV: dorsal motor nucleus of the vagus, sol: solitary tract, 12: hypoglossal nucleus. C: The time course of mean swallowing frequency (left) and mean latency (right) during electrical stimulation of the SLN is shown. The injection of GLP-1 into the M-DVC but not L-DVC decreased the frequency of reflex swallowing and increased the latency of the first swallow. Asterisks indicate significant differences compared with the value 5 min before drug injection by Dunnett's test ($P < 0.05$).