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Original Paper

Effects of NK_1 and NMDA Receptor Antagonists on Severe Gagging Induced by Superior Laryngeal Nerve Stimulation in Rats

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Key words: severe gagging, NK_1 receptor, NMDA receptor, superior laryngeal nerve, decerebration

Abstract

Severe gagging, which is considered to be one of the emetic responses, prevents food bolus from being lodged in the pharyngo-laryngeal region. Little is known about the neuronal mechanism of severe gagging, because severe gagging is difficult to induce in animal experiments. In a recent study the authors reported that pulse train stimulation of the superior laryngeal nerve (SLN) at high frequency can be used to induce severe gagging in decerebrate rats. The present study was focused on the roles of NK_1 and NMDA receptors in severe gagging and the influence that the receptor antagonists may exert on this process. Intravenous administration of WIN51708, NK_1 receptor antagonist, significantly diminished the probability of the induction of severe gagging elicited by 3 sets of pulse train of the SLN at intervals of 1.7 sec (100Hz, 0.3ms, 50pulses, n=7). Similarly, MK-801, NMDA receptor antagonist, significantly diminished the probability of the induction of severe gagging (n=9). These results suggest that the neural circuits of severe gagging elicited by SLN stimulation could involve both NK_1 and NMDA receptors. Possible action sites of their antagonists in medulla oblongata were discussed and compared with other emetic responses.

Introduction

Pharyngeal constriction elicited by irritation of oral posterior mucosa is called the gag reflex (mild gagging). The reflex is considered as a defense reflex that prevents food bolus from entering the pharynx. More strong and long-lasting stimulation of oral posterior mucosa and/or pharynx elicits simultaneous contraction of diaphragm and abdominal muscle, i.e. severe gagging. A rapid increase in the internal pressure of the stomach is produced by the simultaneous contraction of these muscles, and the pressure spreads to the upper esophagus. Thus, the role of severe gagging is known to prevent food bolus from being lodged in the pharyngo- laryngeal region and the upper esophagus.

Concern about clinical problems related to severe gagging has been growing in recent years. Severe gagging creates many problems when feeding children and performing routine dental treatment [1-3]. According to data reported by the Ministry of Health, Labour and Welfare in Japan, more than 4000 people died due to food bolus impaction in 2006.

The main role of severe gagging is considered to be the expulsion of food bolus from the upper airway. However, little is known about the neural mechanism of severe gagging. Pressing against the oropharyngeal mucosa using a spatula is usually employed to induce mild gagging. Pressing the

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oropharyngeal mucosa strongly elicits severe gagging, which is characterized in humans by a burst discharge of the abdominal muscles and a wide opened mouth [4]. However, it is difficult to adequately equalize the intensity of the pressure and, furthermore, to elicit severe gagging repeatedly using this method. Thus an alternative method for severe gagging induction is preferable. We have recently reported severe gagging can be elicited repeatedly by using high intensity and/or high frequency stimulation of the superior laryngeal nerve (SLN), which innervates the pharyngo-laryngeal region, in decerebrate rats [5]. Furthermore, the induction probability of severe gagging can be controlled by stimulus parameters of the SLN [5]. Afferents of the SLN are known to relay to the nucleus of the solitary tract in the medulla oblongata. It is reported that the command of motor pattern in severe gagging is produced by reticular neurons located dorsomedial to the retrofacial nucleus in the medulla oblongata [6, 7]. However, what types of neurotransmitters or neural receptors are working in severe gagging has not been identified.

Severe gagging is considered to be one of the emetic responses. In the vomiting reflex, one of the emetic responses, tachykinin NK_1 receptors [7-10] and N-methyl-D-aspartate (NMDA) type glutamate receptors [11, 12], play crucial roles in the neuronal circuit of the vomiting reflex. In this study, WIN51708 and MK-801 were selected as the NK_1 and NMDA receptor antagonists, respectively, since these antagonists are known to cross the blood brain barrier (BBB) which protects the brain from changes in the intravascular levels of ions, amino acids, peptides, and other substances. This study aimed to identify neurotransmitters involved in the neural circuit of severe gagging elicited by SLN stimulation in decerebrate rats in order to deepen our understanding of the neuronal mechanisms of severe gagging.

Materials and Methods

The experimental procedures were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science (Physiological Society of Japan, 2003). These procedures were approved by the Institutional Animal Care and Use Committee in Kawasaki University of Medical Welfare (No. 08-018).

Surgical procedure

Experiments were performed on Male-Sprague Dawley rats (weighing from 350 to 500g) that were anesthetized with an intraperitoneal injection of a mixture of urethane (0.7g/kg) and α-chloralose (0.06g/kg). When necessary, supplementary doses of a mixture of urethane (0.12g/kg) and α-chloralose (0.01g/kg) were given. Rectal temperature was maintained at 37-38 °C with a heating pad. A polyethylene cannula was inserted into the trachea to maintain the patency of the respiratory tract. Rats were fixed in a stereotaxic frame in the prone position, the parietal bones were removed, the dura was incised, and precollicular decerebration was performed. The decerebration procedure was performed in order to eliminate cerebral function by transecting the brain at the midbrain level. No further anesthetics were given after the decerebration. The rats were then taken out of the frame and rotated to the supine position. Blood pressure of the femoral artery was monitored. Normal saline was continuously infused through the femoral vein at a rate of 1.5ml/h. The left superior laryngeal nerve (SLN) was dissected free from surrounding tissues and placed on bipolar electrodes.

Stimulation and recording

Severe gagging was elicited using three sets of pulse train stimulation of the SLN at 100Hz (interval: 1.7 sec, intensity: 200-400 μA, pulse number: 10-50 pulses, duration: 0.3 ms). Electromyographic activities (EMGs) of abdominal, suprahyoid and intercostal muscles were recorded using each bipolar tungsten electrode. Severe gagging was identified by the activity patterns of these muscles. The EMG of suprahyoid muscle was used to distinguish severe gagging from the swallowing reflex. Respiration cycles were monitored using the EMG of the intercostal muscles. All data were converted to digital format using an analog-to-digital converter (PowerLab, AD Instruments, Japan), and stored in a computer (Optiplex GX520, DELL, Japan). The digitized data were analyzed using a software program (Chart ver. 5.0, AD Instruments, Japan).

Drug administration

Non-peptide NK_1 receptor antagonist, WIN51708 (1-2 mg/kg, SIGMA, Japan) was dissolved in Dimethyl Sulfoxide. Non-competitive NMDA receptor antagonist, MK-801 (1 mg/kg, SIGMA, Japan) was dissolved in normal saline. Drug administration was performed through the femoral vein.

Statistical analysis

Data was analyzed by chi-square test. Post-hoc test was performed using the test of the difference between two proportions with Ryan's procedure [13]. Significance level was taken as $p< 0.05$.

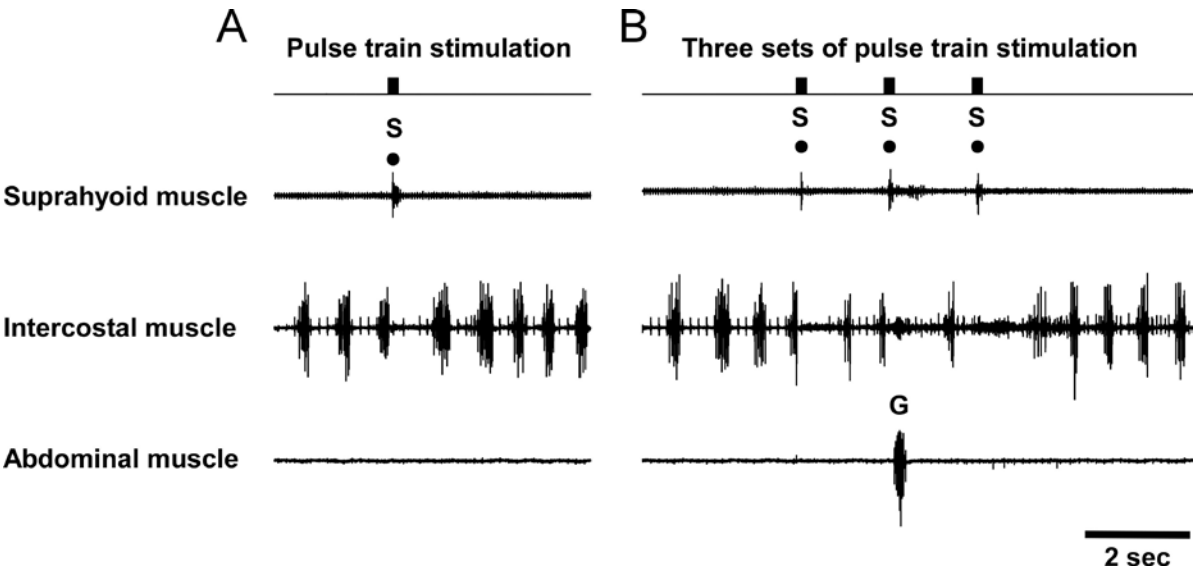

Fig. 1 Typical EMGs of suprahyoid, intercostal and abdominal muscles induced by pulse train simulation of the superior laryngeal nerve (SLN). Single pulse train simulation elicited burst activity of suprahyoid muscle, corresponding to swallowing movement (indicated by "S"). Three sets of pulse train stimulation produced burst activity of abdominal muscle, corresponding to severe gagging (indicated by "G").

Results

Severe gagging and the swallowing reflex elicited by SLN stimulation

Pulse train stimulation of the SLN (100 Hz, 200-400 μA, 10-50 pulses) elicited the swallowing reflex, which was characterized by burst activity of the suprahyoid muscle and transient inhibition of intercostal muscle activity (indicated by "S", Fig. 1A). When three sets of pulse train stimulation were applied to the SLN at 1.7 sec intervals, severe gagging, which was characterized by burst activity of the abdominal muscle, was elicited (indicated by "G", Fig. 1B).

Effects of NK_1 and NMDA receptor antagonists on severe gagging

As described in the introduction, NK_1 and/or NMDA receptors have been reported to be essential to elicit emetic responses such as retching and vomiting. Therefore in this study, the effects of NK_1 receptor antagonist (WIN51708) and NMDA receptor antagonist (MK-801) on severe gagging were examined by intravenous administration of these drugs.

Before intravenous administration of both antagonists, severe gagging was elicited by three sets of pulse train stimulation of the SLN in all rats. Distinct changes in blood pressure and respiratory cycle were not observed after administration of both antagonists. The administration of WIN51708 gradually diminished the probability of the induction of severe gagging and severe gagging was not elicited at 14 min after the administration (Fig. 2). Severe gagging was elicited by SLN stimulation 60 min after the administration of WIN51708 in all rats. The probability of the induction of severe gagging significantly decreased at 10 and 14 min after the administration of WIN51708 when compared with pre-administration results (chi-square test and post-hoc test, n=7, $p<0.05$; Fig. 2). Similarly, the administration of MK-801 diminished the

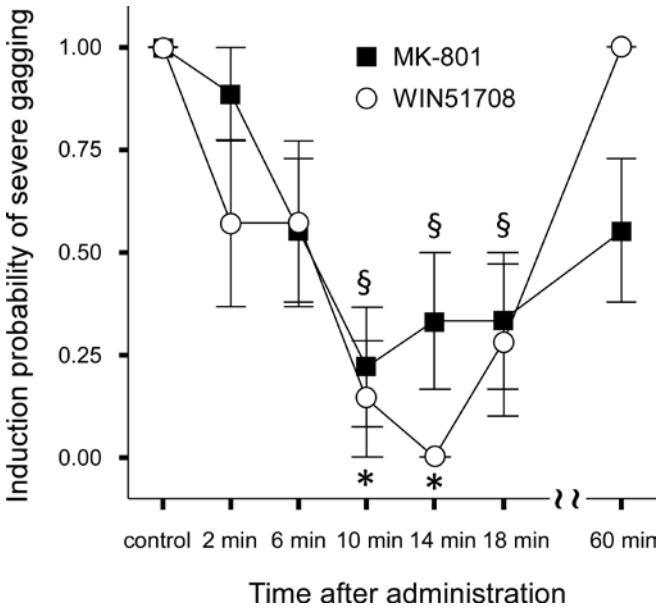

Fig. 2 Effects of NK_1 and NMDA antagonists on induction probability of severe gagging elicited by SLN stimulation. Values are presented by means ± S.E. Pretreatment of WIN51708 (n=7) and MK-801 (n=9) significantly diminished induction probability of severe gagging. Each significance level ($p<0.05$) is showed by * and §.

probability of the induction of severe gagging and it reached a minimum value at the 10 min point after administration (Fig. 2). However, the induction probability of severe gagging did not recover to the control level even 60 min after the administration. The probability of the induction of severe gagging significantly decreased at 10, 14 and 18 min after the administration of MK-801 (chi-square test and post-hoc test, n=9, $p<0.05$; Fig. 2).

Discussion

Effect of NK_1 receptor antagonist on severe gagging

In the present study, intravenous administration of NK_1 receptor antagonist WIN51708 significantly suppressed severe gagging. Distinct changes in blood pressure and respiratory cycle were not observed after administration of WIN51708 (1-2mg/kg). Synaptic transmission in the neural circuit of severe gagging seemed to be inhibited by the drug without it influencing the respiratory and circulatory center in medullary reticular formation. This drug is reported to cross the blood brain barrier [14], thus, since precollicular decerebration was performed in this study, the binding site of WIN51708 would have been within the brainstem. Therefore we must ask the question, at which location did WIN51708 act to diminish severe gagging? The superior laryngeal nerve (SLN), whose stimulation was used for the induction of severe gagging, projects the nucleus of the solitary tract (NST) in the medulla oblongata. Therefore, NST seems to be one candidate for the biding sites of WIN51708. In this study, the SLN was stimulated at a high frequency and a relatively high intensity, which was considered as noxious stimulation. Thus, it is possible that SLN stimulation activated unmyelinated fibers contained in the SLN and that release of substance P (SP) from these fibers was facilitated. The basis of this discussion is as follows; NK_1 receptor has a high affinity for SP, a tachykinin peptide. The neurotransmitter consists of 11 amino-acid residues and is localized in unmyelinated slow conducting C-fibers [15]. The SP is known to be a neurotransmitter involved in emetic responses [8, 9] and wind-up pain [16]. Severe gagging is usually elicited by noxious stimulation in the pharynx and larynx due to food impaction or irritation of mucosa in this area. Taken together, the NK_1 receptor antagonists, WIN51708 might interrupt neural transmission from C-fibers of the SLN to secondary neurons of the NST.

On the other hand, it is reported that the vomiting reflex, which is a typical emetic response, is elicited via the NK_1 receptor in the medullary area adjacent to the semicompact part of the nucleus ambiguus [7, 17]. Aprepitant is a NK_1 receptor antagonist, which has Food and Drug Administration (FDA) approval, and is widely used as antiemetic medicine in clinical fields. Severe gagging is considered to be one of the emetic responses. Therefore, it is possible that WIN51708 acts at the medullary area adjacent to the semicompact part of the nucleus ambiguus, where it is known to be activated by NST neurons [7]. Thus, the precise locations of the effective sites of WIN51708 remain unknown and further study using microinjection of the receptor antagonists should be performed in the future to resolve this issue.

In contrast to this study's findings, Watson et al. [8] reported that the NK_1 receptor antagonist did not affect "mild" gagging which is characterized by pharyngeal constriction elicited by tactile stimulation of the pharyngeal mucosa in conscious ferrets. Discrepancies between Watson et al.'s results and the present study may well be differences between "mild" and "severe" gagging. Induction of severe gagging necessitates high frequency stimulation of the SLN. As described above, the noxious stimulation should release SP, however SP would not be a causal agent in "mild" gagging elicited by tactile stimulation of the pharyngeal mucosa. In addition, conscious ferrets were used in their study, whereas decerebrate rats were used in our study. Gagging is one of the medullary reflexes and is influenced by inputs of the higher

nervous system such as the cerebral cortex and limbic system. It is reported that gagging is facilitated by psychological factors such as certain smells, sounds and emotions [1, 18]. This may also be a reason for the discrepancies between the two studies.

Effect of NMDA receptor antagonist on severe gagging

Pretreatment of the NMDA receptor antagonist, MK-801 significantly diminished the induction of severe gagging. MK-801 (1mg/kg) was intravenously administered, however distinct changes in blood pressure and respiratory cycle were not observed. This drug is known to penetrate the blood brain barrier [19], thus, since precollicular decerebration was performed, the binding site of MK-801 would have been within the brainstem.

Glutamate is a major excitatory neurotransmitter in the brain and spinal cord. NMDA receptor is an ionotropic class of glutamate receptor. NMDA receptors do not participate in normal synaptic transmission because of their voltage-dependent block by extracellular magnesium [20]. A repetitive drive of noxious afferents such as C-fibers decreases the magnesium block and channel opening time is prolonged [20]. NMDA receptors have received particular attention because of their crucial roles in excitatory synaptic transmission and plasticity such as wind-up pain and memory consolidation [20]. The high frequency electrical stimulation of the SLN applied in this study is considered as noxious in the pharyngo-laryngeal region. Therefore, it is possible that SLN stimulation strongly activated C-fibers, and as a result, the magnesium blockade in the secondary neurons in the NST was released. The results obtained by our experiment indicated that NMDA receptors in NST neurons might participate in a neural circuit of severe gagging. In fact, many studies have indicated that vomiting reflex is inhibited by intravenous or subcutaneous administration of NMDA receptor antagonists such as CGS19755 and dextromethorphan in ferrets and cats [10-12].

Our previous study showed that emetic stimulations, e.g., emetic drug administration or hypoxia, promoted the induction of severe gagging [4, 21]. Conditioned taste aversion (CTA) is a well-known emetic response observed in rats. CTA is defined as the result of the association of a taste with gastrointestinal malaise produced by the intake of a toxin and it is considered as an alternative strategy for the vomiting reflex in rats. It is reported that CTA is also inhibited by the intraperitoneal administration of an NMDA receptor antagonist, ketamine [22]. Moreover, we have recently reported that autonomic responses (e.g., relaxation of gastric fundus and decrease of blood pressure) induced in rats by emetic drugs were inhibited by the intravenous administration of MK-801 [5]. These studies strongly support that NMDA receptors mediate emetic responses such as CTA and autonomic signs associated with emesis. Thus, we concluded that NMDA receptors participate in the induction of severe gagging.

On the other hand, Furukawa et al. [23] have reported that intraventricular administration of the non-NMDA receptor antagonist (NBQX), (but not NMDA receptor antagonist MK-801), significantly depressed retching and salivary secretion elicited by abdominal vagal afferents stimulation in decerebrate dogs. They concluded that non-NMDA receptors in NST participated in the neural circuit of these emetic responses, since fourth ventricular injection of the drug would directly act on NST neurons. Thus, non-NMDA and NMDA receptors might be involved in emetic responses induced by vagal afferent and SLN stimulation, respectively. This assumption should be further investigated.

Interaction between NK_1 and NMDA receptors

In this study, it was suggested that NK_1 and NMDA receptors are involved in the neural circuit of severe

gagging. However, the action sites of NK_1 and NMDA receptors remained uncertain, because antagonists of these receptors were administered intravenously. Thus, it seems to be impossible to investigate the interaction between the NK_1 and NMDA receptors. The interaction between NK_1 and NMDA receptors in association with severe gagging should be discussed in a future microinjection study.

Conclusion

This study aimed to identify neuronal receptors involved in severe gagging elicited by SLN stimulation in decerebrate rats. Intravenous administration of WIN51708 or MK-801 significantly diminished the probability of the induction of severe gagging. It is concluded that both NK_1 and NMDA receptors could be involved in neural circuit of severe gagging.

References

1. Bassi GS, Humphris GM, Longman LP: The etiology and management of gagging: a review of the literature. *J Prosthet Dent* 91:459-467, 2004.
2. Scarborough DR, Boyce S, McCain G, Oppenheimer S, August A, Strinjas JN: Abnormal physiological responses to touch among children with persistent feeding difficulties. *Dev Med Child Neurol* 48:460-464, 2006.
3. Barenboim SF, Dvoyris V, Kaufman E: Does granisetron eliminate the gag reflex? A crossover, double-blind, placebo-controlled pilot study. *Anesth Prog* 56:3-8, 2009.
4. Ashida C, Koga T: Effects of arterial oxygen saturation on gag reflex in humans. *Kawasaki J Med Welfare* 11:63-68, 2006.
5. Yamagata R, Himi N, Koga T: Emetic responses are mediated via NMDA and/or NK_1 receptors in decerebrate rats. *The Journal of Physiological Sciences* 59:476, 2009.
6. Fukuda H, Koga T: Neuronal gagging activity patterns may be generated by neurons in the reticular area dorsomedial to the retrofacial nucleus in dogs. *Exp Brain Res* 113:394-401, 1997.
7. Fukuda H, Koga T, Furukawa N, Nakamura E, Hatano M, Yanagihara M: The site of the antiemetic action of NK_1 receptor antagonists, in *Antiemetic Therapy*, edited by Donnerer J, Basel, Karger, 2003, pp33-77.
8. Watson JW, Gonsalves SF, Fossa AA, McLean S, Seeger T, Obach S, Andrews PL: The anti-emetic effects of CP-99,994 in the ferret and the dog: role of the NK_1 receptor. *Br J Pharmacol* 115:84-94, 1995.
9. Fukuda H, Koga T, Furukawa N, Nakamura E, Shiroshita Y: The tachykinin NK_1 receptor antagonist GR205171 abolishes the retching activity of neurons comprising the central pattern generator for vomiting in dogs. *Neurosci Res* 33:25-32, 1999.
10. Saito R, Takano Y, Kamiya H: Roles of substance P and NK_1 receptor in the brainstem in the development of emesis. *Journal of Pharmacological Sciences* 91:87-94, 2003.
11. Lehmann A, Kärrberg L: Effects of N-methyl-D-aspartate receptor antagonists on cisplatin-induced emesis in the ferret. *Neuropharmacology* 35:475-481,1996.
12. Lucot JB: Effects of N-methyl-D-aspartate antagonists on different measures of motion sickness in cats. *Brain Res Bull* 47:407-411, 1998.
13. Ryan TA: Significance tests for multiple comparison of proportions, variances, and other statistics. *Psychol Bull* 57:318-328, 1960.
14. Lazareno S, Popham A, Birdsall NJ: Analogs of WIN 62,577 define a second allosteric site on muscarinic receptors. *Mol Pharmacol* 62:1492-1505, 2002.
15. Goto T, Kido MA, Yamaza T, Tanaka T: Substance P and substance P receptors in bone and gingival tissues. *Med Electron Microsc* 34:77-85, 2001.
16. Girish C, Manikandan S: Aprepitant: a substance P antagonist for chemotherapy induced nausea and vomiting.

Indian J Cancer 44:25-30, 2007.

17. Onishi T, Mori T, Yanagihara M, Furukawa N, Fukuda H: Similarities of the neuronal circuit for the induction of fictive vomiting between ferrets and dogs. *Auton Neurosci* 136:20-30, 2007.
18. Van Linden van den Heuvell GF, Ter Pelkwijk BJ, Stegenga B: Development of the gagging problem assessment: a pilot study. *J Oral Rehabil* 35:196-202, 2008.
19. Liu JX, Du JZ, Asai S, Shi ZQ, Watanabe G, Taya K: NMDA receptor antagonists reduce restraint-induced release of prolactin in male rats. *Neuro Endocrinol Lett* 24:435-439, 2003.
20. Petrenko AB, Yamakura T, Baba H, Shimoji K: The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. *Anesth Analg* 97:1108-1116, 2003.
21. Kurozumi C, Yamagata R, Himi N, Koga T: Emetic stimulation inhibits the swallowing reflex in decerebrate rats. *Auton Neurosci* 140:24-29, 2008.
22. Traverso LM, Ruiz G, Camino G, De la Casa LG: Ketamine blocks the formation of a gustatory memory trace in rats. *Pharmacol Biochem Behav* 90:305-311, 2008.
23. Furukawa N, Hatano M, Fukuda H, Koga T: Non-N-methyl-D-aspartate receptors may mediate the transmission of emetic signals between visceral vagal afferents and the solitary nucleus in dogs. *Neurosci Lett* 258:53-56, 1998.