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研究課題名（和文）Elucidating Novel Therapeutic Targets for Oropharyngeal Dysphagia: Focusing on TRPA1 and TRPV4 Channels

研究課題名（英文）Elucidating Novel Therapeutic Targets for Oropharyngeal Dysphagia: Focusing on TRPA1 and TRPV4 Channels

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研究成果の概要（和文）：本研究は、嚥下反射におけるTRPA1とTRPV4の関与を明らかにすることである。これまでの実験結果により、TRPA1は細胞体の大きさが小型から中型のニューロンに局在するのに対し、TRPV4は大型から中型のニューロンに局在することが判明した。TRPA1またはTRPV4のアゴニストを咽頭・喉頭領域に局所投与すると、用量依存的に嚥下反射が促進した。さらに、TRPA1またはTRPV4 のアンタゴニストを事前投与すると、アゴニストによる嚥下反射の誘発は抑制された。
これらの結果は、TRPA1とTRPV4 をターゲットとした研究が嚥下機能の低下を改善する治療薬の開発につながる可能性を示唆している。

研究成果の学術的意義や社会的意義
Dysphagia poses a significant health challenge, with no established pharmacological treatment currently available. By targeting TRPA1 and TRPV4, our study suggests the importance of further exploration and development of therapeutics to address this pressing health issue.

研究成果の概要（英文）：We investigate the involvement of TRPA1 and TRPV4 in initiating the swallowing reflex. Our observations reveal that TRPA1 predominantly localizes on small to medium-diameter neurons, whereas TRPV4 primarily localizes on large to medium-diameter neurons. The topical administration of chemical agonists targeting TRPA1, such as allyl isothiocyanate (AITC), or TRPV4, such as GSK1016790A, in swallowing-related regions, leads to a dose-dependent facilitation of the swallowing reflex. Furthermore, the pre-application of antagonists for TRPA1 or TRPV4 significantly mitigates the AITC or GSK1016790A-induced swallowing reflex, respectively. These findings demonstrate the potential of targeting TRPA1 and TRPV4 to develop therapeutics to enhance swallowing function.

研究分野：Oral Physiology

キーワード：Swallowing Reflex　TRPA1　TRPV4

１．研究開始当初の背景

Oropharyngeal dysphagia (difficulty in swallowing) is a major health problem among older people, especially in Japan. It may lead to severe complications, such as pulmonary aspiration, malnutrition, dehydration, and pneumonia. Reduced oropharyngeal sensitivity is one of the major causes of oropharyngeal dysphagia that leads to delayed evoking of the swallowing reflex, and thus, it is highly desirable to find strategies to facilitate the evoking of the swallowing reflex. Increasing the sensory inputs from the swallowing-related regions using chemical stimulants can be a pharmacological therapeutic strategy to improve swallowing functions in patients with oropharyngeal dysphagia. However, understanding the underlying molecular targets of the chemical stimulants and their efficacy is essential for developing effective pharmacological therapeutics. Recently, we observed that activation of TRPV1, TRPM8, and ASIC3 in the swallowing-related regions facilitated the triggering of the swallowing reflex (Hossain et al. 2019, 2018). These observations raise the potentiality of the contribution of other TRP channels (like TRPA1 and TRPV4) in triggering the swallowing reflex. A detailed understanding of the contributions of TRPA1 and TRPV4 in swallowing reflexes is necessary to target these channels for developing pharmacological therapeutics or swallowing-friendly foods/drinks.

２．研究の目的

In this study, our objectives were: 1) Exploring the expression pattern of TRPA1 and TRPV4 in the peripheral swallowing-related regions and ganglia. 2) Exploring the effect of pharmacological activation of TRPA1 and TRPV4 in the triggering of the swallowing reflex. 3) Exploring the effect of inactivation of TRPA1 and TRPV4 in the triggering of the swallowing reflex.

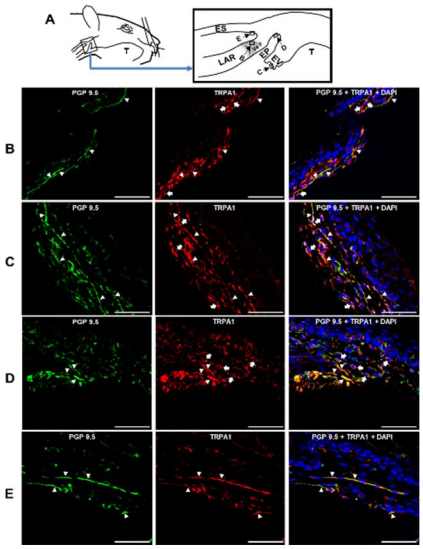

Figure 1. TRPA1 localization in peripheral swallowing-related regions.

３．研究の方法

1) **Experimental Animals:** Sprague Dawley rats. Matsumoto Dental University Intramural Animal Care and Veterinary Science Committee approved the experimental procedures. We adhered to the guidelines of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research, ARRIVE (Animal Research: Reporting of In Vivo Experiments). 2) **Immunohistochemistry and RT-PCR:** Fluorescent immunohistochemistry was conducted to detect TRPA1 and TRPV4 in the superior laryngeal nerve (SLN)-innervated peripheral swallowing-related regions and in the nodose-petrosal-jugular ganglionic complex (NPJc) which contains cell bodies of SLN-afferent neurons. RT-PCR was conducted to detect TRPA1 and TRPV4 in the NPJc. 3) **Swallowing reflex recording:** Electromyogram (EMG) activity of the mylohyoid muscle was recorded to identify the swallowing reflex. The bilateral SLNs

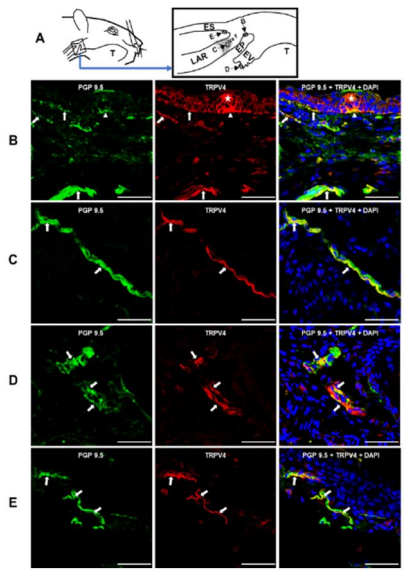

Figure 2. TRPV4 localization in peripheral swallowing-related regions.

were left intact, but the pharyngeal (IX-ph) and lingual (IX-li) branches of the glossopharyngeal, pharyngeal (X-ph), and recurrent laryngeal (RLN) branches of the vagus nerve were transected bilaterally to avoid the influence of non-SLNs in triggering the swallowing reflex.

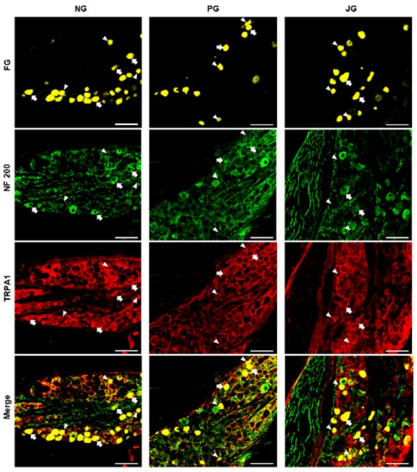

Figure 3. TRPA1 localization in NPJc.

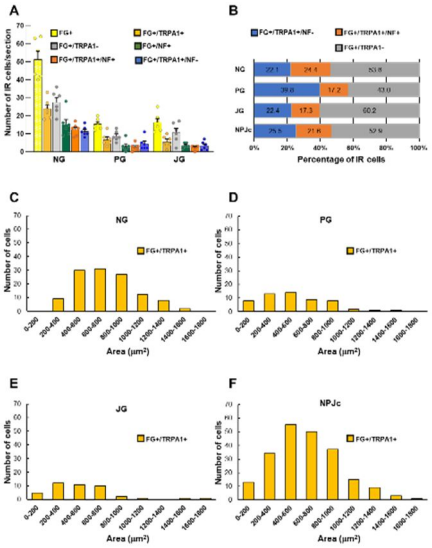

Figure 4. Distribution of TRPA1 localization in the NPJc.

4) **Stimulating solutions:** The stimulating solutions were saline, various concentrations of a TRPA1 agonist, AITC; various concentrations of a TRPV4 agonist, GSK1016790A. 5) **Antagonists:** a TRPA1 antagonist, HC-030031 and a TRPV4 antagonist, RN-9893 were used. 6) **Data and statistical analysis:** The triggered swallowing reflexes were counted for 20 s after applying the stimulating solutions. Additionally, the average interval between the triggered swallowing reflexes was calculated from the reflexes evoked within 10 s following solution delivery. For statistical analysis, data were checked by normality and equal variance tests to determine whether to run parametric or non-parametric tests. The number of the swallowing reflexes triggered by different concentrations of of the TRPA1 or TRPV4 agonists and the number and intervals of the reflexes with and without prior application of the respective antagonists or vehicle were compared using one-way repeated measures analysis of variance (ANOVA) followed by Tukey's test. The intervals of the swallowing reflexes were compared using Friedman repeated measures ANOVA on ranks followed by Tukey's test. The numbers of agonists-triggered swallowing reflexes with and without prior application of lidocaine or transection of SLNs were compared using a paired t-test. Differences were considered significant at $P < 0.05$. The data are shown as the mean ± SEM.

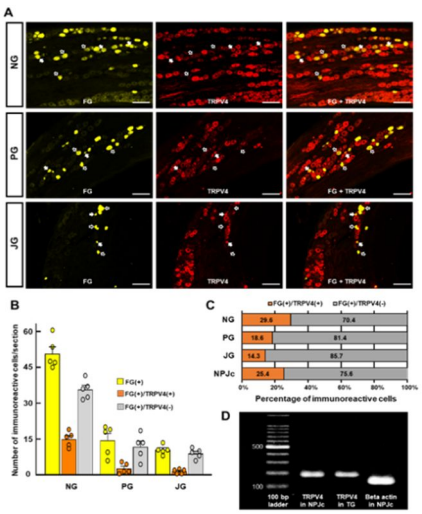

Figure 5. TRPV4 localization in NPJc.

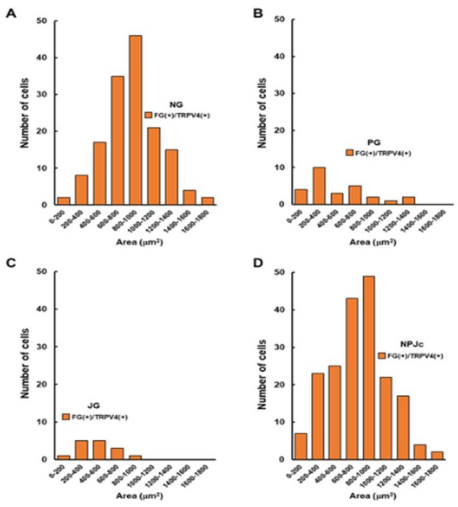

Figure 6. Distribution of TRPV4 localization in the NPJc.

4．研究成果

1) TRPA1 and **TRPV4 are expressed in the peripheral swallowing-related regions**

We examined whether TRPA1 and TRPV4 are localized on nerve fibers in SLN-innervated swallowing-related regions. Nerve fibers were detected using a nerve-fiber marker, PGP 9.5. We observed TRPA1 and TRPV4 on some PGP 9.5-expressing nerve fibers in SLN-innervated swallowing-related regions (Figure 1 and 2).

2) SLN-afferent neurons in the NPJc expressed TRPA1 and TRPV4

TRPA1 and TRPV4 localized on SLN-afferent neurons in the NPJc (Figure 3 to 6). SLN-afferent neurons in the NPJc were traced using FG (a retrograde tracer). Figure 4 shows the number, percentage and distribution of TRPA1-positive FG-stained SLN-afferent neurons in NPJc. Figure 5 and 6 show the number, percentage and distribution of TRPV4-positive FG-stained SLN-afferent neurons in NPJc. We also observed TRPV4 mRNA in the NPJc using RT-PCR (Figure 5).

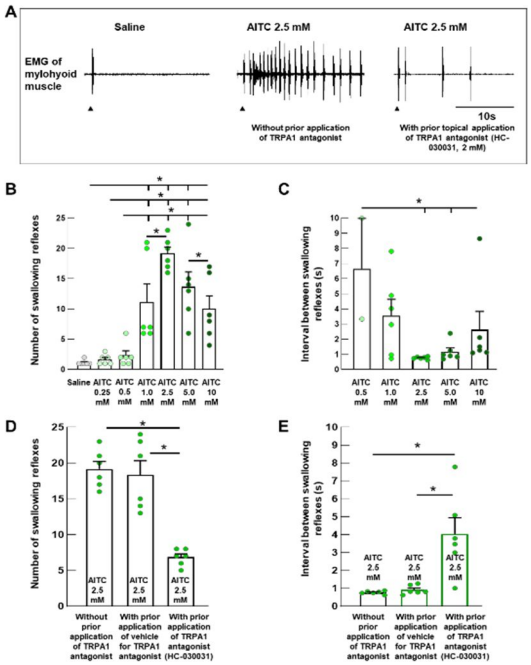

Figure 7. Topical application of AITC, a chemical agonist of TRPA1s triggered the swallowing reflexes which were significantly attenuated by prior topical application of a TRPA1 antagonist.

3) Topical application of TRPA1 and TRPV4 agonists, facilitated triggering of the swallowing reflex

Next, we examined whether activation of TRPA1 and TRPV4 in swallowing-related regions can trigger the swallowing reflex. Different concentrations of a potent TRPA1 agonist (allyl isothiocyanate, AITC) and a TRPV4 agonist (GSK1016790A) were topically applied to the SLN-innervated swallowing-related regions. Saline or vehicle for the agonists were also applied to the regions that triggered only one or two swallowing reflexes (Figures 7 and 8). AITC and GSK1016790A application triggered swallowing reflexes in a dose-dependent manner (Figures 7 and 8). Additionally, increasing the concentration of AITC and GSK1016790A decreased the intervals between the triggered

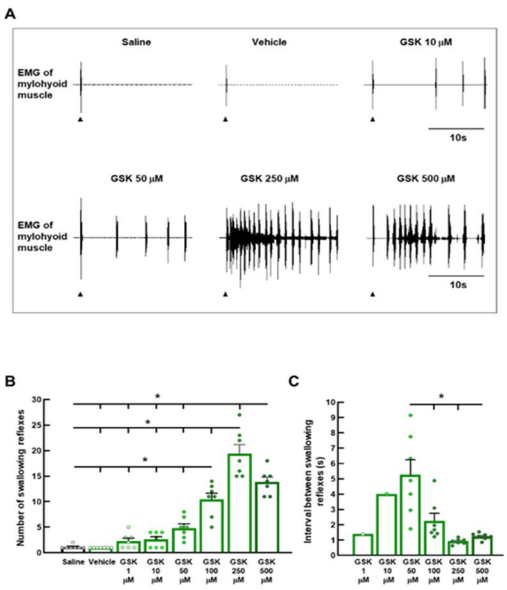

Figure 8. Topical application of GSK1016790A, a TRPV4 agonist, triggered swallowing reflexes in a dose-dependent manner.

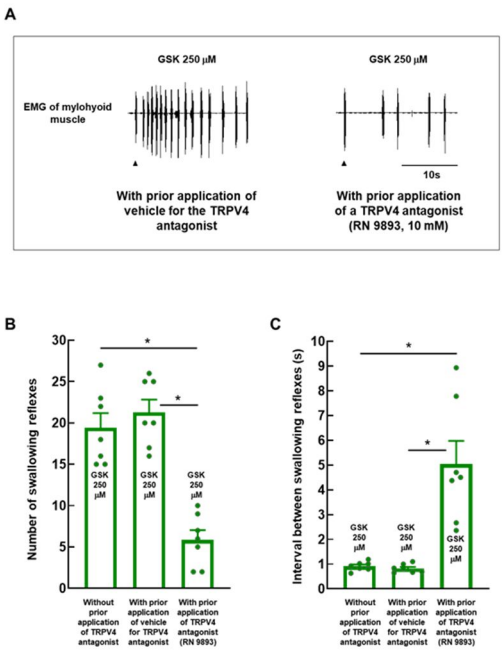

Figure 9. Topical application of RN-9893, a TRPV4 antagonist, prior to the application of GSK1016790A, significantly reduced the number of GSK1016790A-triggered swallowing reflexes.

reflexes (Figures 7 and 8).

4) TRPA1 and TRPV4 antagonists significantly reduces the number of agonists-induced swallowing reflexes

The number of swallowing reflexes induced by AITC was significantly reduced by prior topical application of a TRPA1 antagonist, HC-030031 (Figures 7D). Additionally, the number of swallowing reflexes induced by GSK1016790A was significantly reduced by prior topical application of a TRPV4 antagonist, RN-9893 (Figures 9). The vehicle for the antagonist had no significant effect on the number of AITC or GSK1016790A-induced swallowing reflexes (Figures 7 and 9). The intervals between the triggered reflexes were significantly increased by the TRPA1 and TRPV4 antagonist (Figures 7 and 9).

◎考察

The above findings suggest that that TRPA1 and TRPV4 are present on the afferent nerves innervating the peripheral swallowing-related regions and are functional in triggering the swallowing reflex. TRPA1 and TRPV4 can be pharmacologically targeted to facilitate swallowing to manage oropharyngeal dysphagia.

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３．雑誌名 Japanese Dental Science Review	６．最初と最後の頁 421～430
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jdsr.2023.09.002	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

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３．雑誌名 Frontiers in Cellular Neuroscience	６．最初と最後の頁 1149793
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2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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