様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ５ 月 １６ 日現在

機関番号：３３６０２
研究種目：研究活動スタート支援
研究期間：2022～2023
課題番号：２２Ｋ２１０２７

研究課題名（和文）Elucidating functional involvement of transient receptor potential vanilloid 4 (TRPV4) to trigger swallowing reflex

研究課題名（英文）Elucidating functional involvement of transient receptor potential vanilloid 4 (TRPV4) to trigger swallowing reflex

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究は、嚥下反射における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、TRPV4免疫反応性神経は上喉頭神経（SLN)支配の嚥下反射領域に存在することが判明した。逆行性神経トレーシングは、SLNの25%にTRPV4の局在を示した。そのうち、49%が大型、35%が中型、15%が小型のニューロンであった。また、TRPV4 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。TRPV4アンタゴニストの事前処置によりアゴニストの嚥下反射は抑制された。
これらの結果は、TRPV4の研究は嚥下機能の低下を改善する治療のターゲットにな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 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 TRPV4 in initiating the swallowing reflex. Our observations reveal that TRPV4 immunoreactive nerve fibers present in the superior laryngeal nerve (SLN)-innervated swallowing-related regions. Retrograde tracing with fluorogold reveals localization of TRPV4 on approximately 25% of SLN-afferent neurons in the nodose-petrosal-jugular ganglionic complex. Among them, approximately 49% are large, 35% medium, and 15% small-sized SLN-afferent neurons. Furthermore, our investigation demonstrates that the topical administration of a chemical agonists targeting TRPV4, GSK1016790A, in swallowing-related regions, leads to a dose-dependent facilitation of the swallowing reflex. Notably, the preapplication of antagonists for TRPV4 significantly mitigates the GSK1016790A-induced swallowing reflex.
These findings underscore the potential of targeting TRPV4 to develop therapeutics to enhance swallowing function.

研究分野：Oral Physiology

キーワード：Swallowing Reflex　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 TRPV4) in triggering the swallowing reflex. A detailed understanding of the contributions of TRPV4 in swallowing reflexes is necessary to target the channel 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 TRPV4 in the peripheral swallowing-related regions and ganglia. 2) Exploring the effect of pharmacological activation of TRPV4 in the triggering of the swallowing reflex. 3) Exploring the effect of inactivation of TRPV4 in the triggering of the swallowing reflex.

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 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 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 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. **4) Stimulating solutions:** The stimulating solutions were saline, various concentrations of a TRPV4 agonist, GSK1016790A. **5) Antagonists:** a TRPV4 antagonist, RN-9893 was 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 TRPV4 agonists and the number and intervals of the reflexes with and without prior application of the TRPV4 antagonist 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.

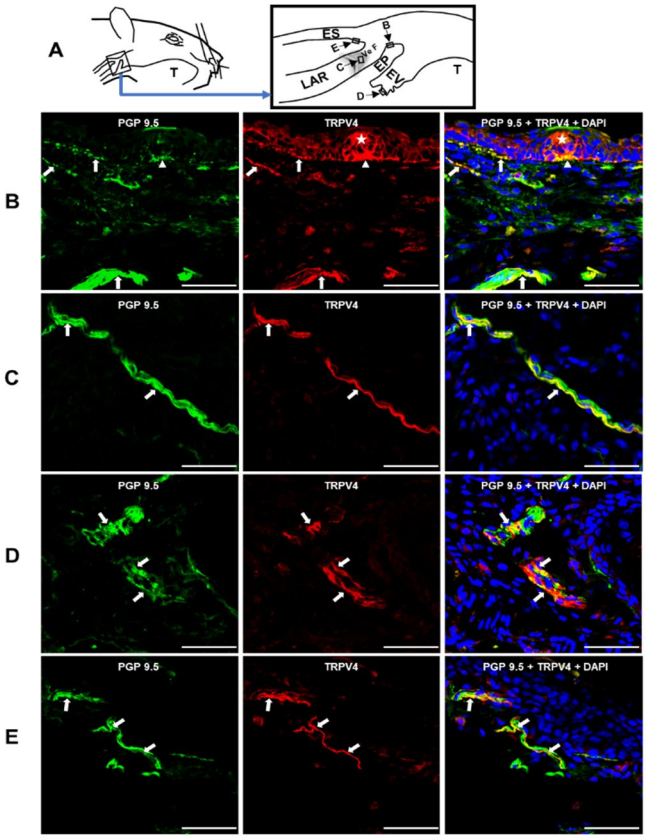

Figure 1. TRPV4 localization in peripheral swallowing-related regions.

4．研究成果

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

We examined whether 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 TRPV4 on some PGP 9.5-expressing nerve fibers in SLN-innervated swallowing-related regions (Figure 1).

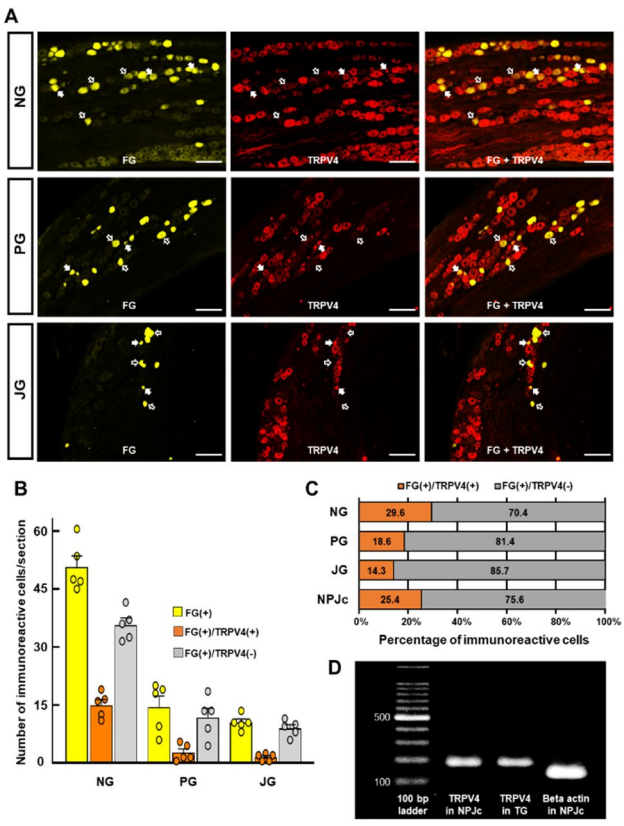

Figure 2. TRPV4 localization in NPJc.

2) SLN-afferent neurons in the NPJc expressed TRPV4

TRPV4 localized on SLN-afferent neurons in the NPJc (Figure 2 and 3). SLN-afferent neurons in the NPJc were traced using FG (a retrograde tracer). Figure 2 shows the number and percentage of TRPV4-positive FG-stained SLN-afferent neurons in NPJc. We also observed TRPV4 mRNA in the NPJc using RT-PCR (Figure 2). Figure 3 shows the distribution of TRPV4-positive FG-stained SLN-afferent neurons in NPJc.

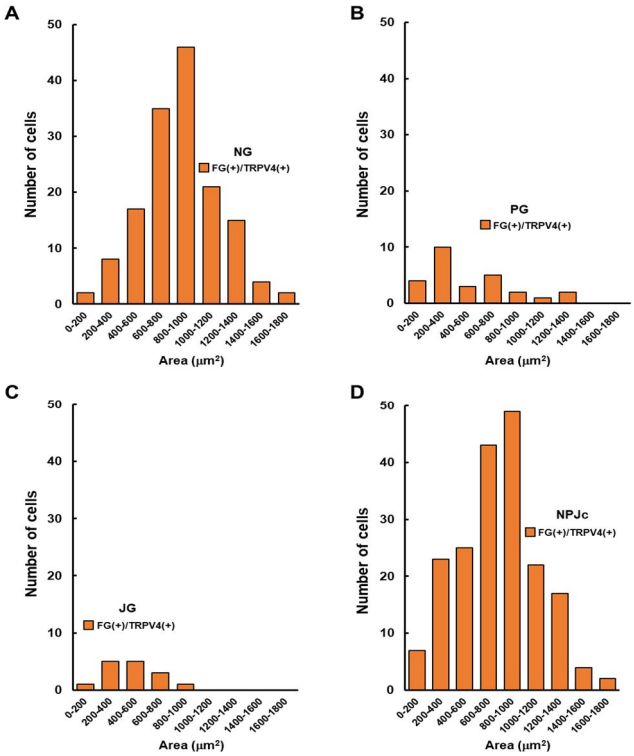

Figure 3. Distribution of TRPV4 localization in the NPJc.

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

Next, we examined whether activation of TRPV4 in swallowing-related regions can trigger the swallowing reflex. Different concentrations of a potent 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 4). GSK1016790A application triggered swallowing reflexes in a dose-dependent manner (Figure 4B). Additionally, increasing the concentration of GSK1016790A decreased the intervals between the triggered reflexes (Figure 4C).

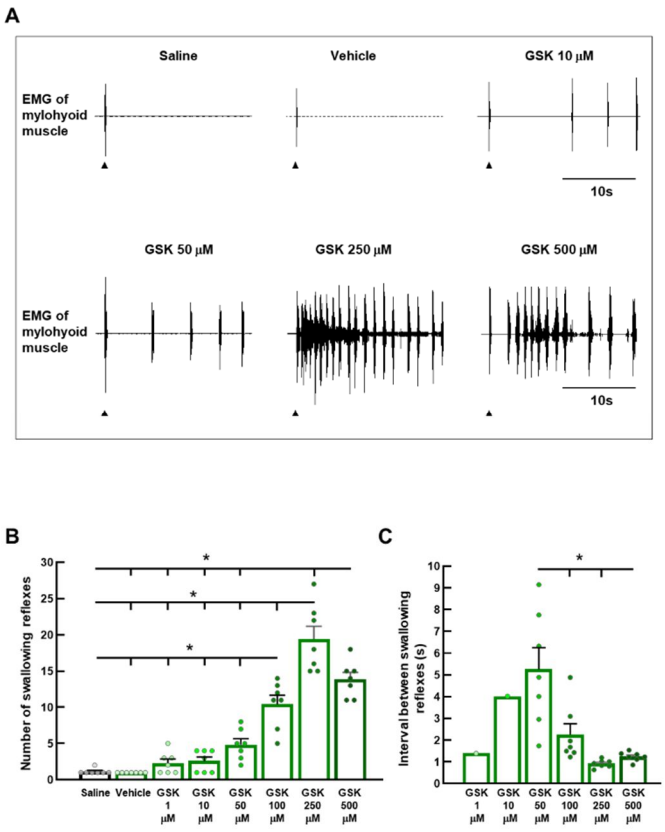

Figure 4. Topical application of GSK1016790A, a TRPV4 agonist, triggered swallowing reflexes in a dose-dependent manner.

4) TRPV4 antagonists significantly reduces the number of agonist-induced swallowing reflexes

The number of swallowing

reflexes induced by GSK1016790A was significantly reduced by prior topical application of a TRPV4 antagonist, RN-9893 (Figure 5). The vehicle for the antagonist had no significant effect on the number of GSK1016790A-induced swallowing reflexes (Figure 5B). The intervals between the triggered reflexes were significantly increased by the TRPV4 antagonist (Figure 5C).

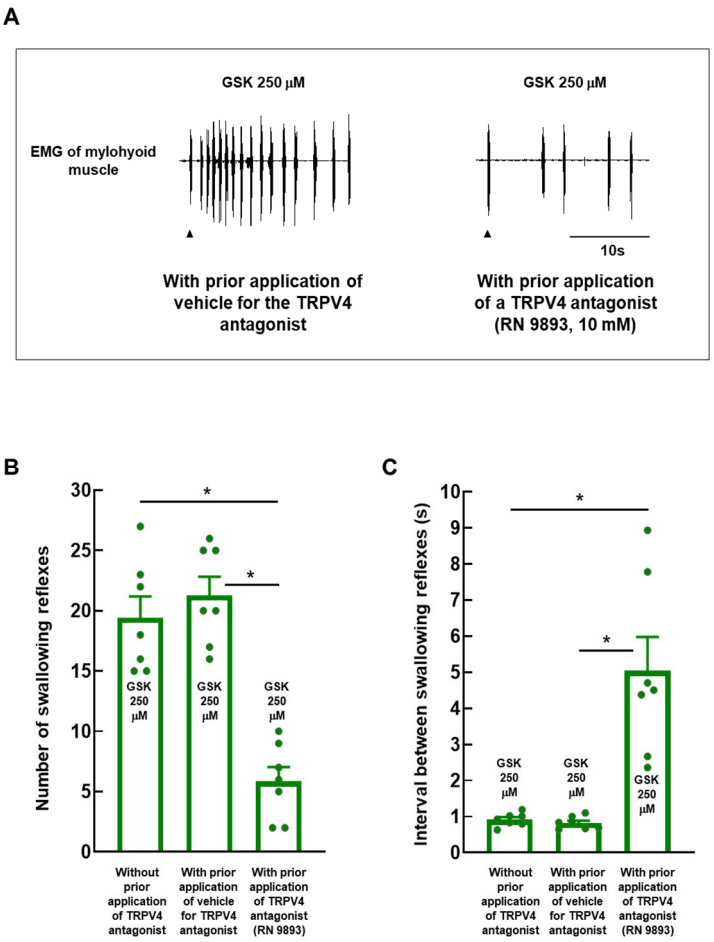

Figure 5. Topical application of RN-9893, a TRPV4 antagonist, prior to the application of GSK1016790A, significantly reduced the number of GSK1016790A-triggered swallowing reflexes.

◎考察

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

◎ 主な発表論文等

〔雑誌論文〕（計 12 件）

1. Hossain MZ, Ando H, Unno S, **Roy RR**, Kitagawa J. (2023) Pharmacological activation of transient receptor potential vanilloid 4 promotes triggering of the swallowing reflex in rats. Frontiers in Cellular Neuroscience 17:1149793. （査読有）
2. Hossain MZ, Kitagawa J. (2023) Transient receptor potential channels as an emerging therapeutic target for oropharyngeal dysphagia. Japanese Dental Science Review 59:421-430. （査読有）

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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　2件）

１．著者名 Hossain Mohammad Zakir、Ando Hiroshi、Unno Shumpei、Roy Rita Rani、Kitagawa Junichi	４．巻 17
２．論文標題 Pharmacological activation of transient receptor potential vanilloid 4 promotes triggering of the swallowing reflex in rats	５．発行年 2023年
３．雑誌名 Frontiers in Cellular Neuroscience	６．最初と最後の頁 1149793
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fncel.2023.1149793	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 －

１．著者名 Hossain Mohammad Zakir、Kitagawa Junichi	４．巻 59
２．論文標題 Transient receptor potential channels as an emerging therapeutic target for oropharyngeal dysphagia	５．発行年 2023年
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 －

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Hossain MZ, 安藤　宏, Roy RR, 海野　俊平, 北川　純一
２．発表標題 Involvement of TRPV4 channel in water-induced swallowing reflex and SLN-response.
３．学会等名 第65回歯科基礎医学会学術大会
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１．発表者名 安藤 宏, Hossain MZ, Roy RR, 海野 俊平, 北川 純一
２．発表標題 上喉頭神経のTRPV4チャネルは水刺激による嚥下反射に関与する
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２．発表標題 Involvement of TRPV4 channels expressed in the afferents of superior laryngeal nerve for triggering swallowing reflex
３．学会等名 第56回日本味と匂学会
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関