Report of „Research Award of Oral Sciences”

Department: Medical Pharmacology
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Title: **Elucidation of the mechanisms underlying the activation of α1-adrenoceptor-induced AQP5 trafficking in rat salivary glands.**

1. Aim of the research and results obtained:

Saliva has a pivotal role in lubrication and protection, maintenance of tooth integrity, buffering action and clearance as well as in taste and digestion. Defective secretion of saliva (hyposalivation) causes dry mouth (xerostomia) and predisposes to severe dental caries and oral mucosal disorders. The activation of muscarinic (M1 and M3) and α1-Adrenergic receptors (ARs) in rat parotid glands is reported to induce secretion of saliva in rats and humans and contribute to more than 50% of total salivary secretions when stimulated. One of the important water transport proteins involved in saliva secretion is aquaporin 5 (AQP5) which has been demonstrated to be highly expressed in parotid glands. Studies with knockout mice lacking AQP-5 show defective fluid secretion, indicating an important role of AQP5 in water secretion in the salivary gland acinar cells. *In vitro* experiments using rat parotid gland slices indicated that acetylcholine (ACh) and epinephrine acting at M3-mAChRs and a1-adrenergic receptors, respectively, induce an increase in AQP5 level in the apical plasma membrane (APM) by increasing Ca2+ concentration. However, it is not known whether a1-adrenoceptor activation induce translocation of AQP5 together with lipid rafts in *in vivo* conditions and which α1-adrenoceptor subtype induces trafficking of AQP5 in rat parotid acinar cells.

In this study, we found that under control conditions, AQP5 and GM1 in parotid acinar cells were distributed in large amounts throughout the cytoplasm and apical plasmalemmal region. However, the activation of a1-adrenoceptor induced trafficking of AQP5 and GM1 to apical and lateral plasma membrane (LPM) and the movement peaked from 6 to 10 min after phenylephrine injection. We also demonstrated that phentolamine inhibited the phenylephrine-induced increase in AQP5 and GM1 in the APM and LPM of rat parotid gland. Additionally, we have discovered that the phenylephrine-induced AQP5 translocation to APM

was inhibited by the a1A-adrenergic specific antagonist silodosin. However, neither a1B-adrenoceptor antagonist L765314 nor a1D-adrenergic receptor antagonist BMY7378, had any significant effect on the amount of AQP5 in the APM. These results were also confirmed by *in vivo* experiment using confocal microscopy, where oral administration of silodosin to rats resulted in inhibition of phenylephrine-induced AQP5 trafficking to APM and LPM. Taken together, above results strongly suggest that phenylephrine acts at a1A-adrenergic receptor to induce the translocation of AQP5 to the APM in rat parotid cells, thus contribute to the saliva secretion.

2. Self-evaluation of research achievement:

The money support from "Research Award of Oral Sciences" was spent on buying reagents which were indispensable to conduct planned experiments. Our research team demonstrated that phenylephrine-induced AQP5 trafficking to apical plasma membrane in rat parotid tissue through a1A-adrenoceptor and therefore contribute to the secretion of saliva. These findings also suggest that silodosin, highly selective a1A-adrenoceptor antagonist, with high tissue selectivity for the salivary glands may enhance the incidence of dry mouth by inhibition of AQP5 trafficking to apical plasma membrane in rat parotid gland. Additional studies will be performed to clarify the involvement of other signal transduction mechanisms underlying the phenylephrine-increase of AQP5 in the APM of rat parotid cells. The manuscript containing the above data is in preparation and is planning to be submitted to the International Journal of Molecular Sciences.

3. Meeting presentation:
Title, conference, venue, date, co-author, presentation (oral/poster).

- "Mechanisms underlying the activation of a1-adrenergic receptor-induced AQP5 trafficking in rat salivary glands", The 11th Tokushima Bioscience Retreat, Shodoshima, Japan, September 17-19, 2015. Aneta Bragiel, Tomasz Pieczonka, Yasuko Ishikawa. (oral)

- "Restoration of physiological age-dependent salivary glands atrophy by whey supplementation in senescent rat model", The 11th Tokushima Bioscience

Retreat, Shodoshima, Japan, September 17-19, 2015. Tomasz Pieczonka, Aneta Bragiel, Kana Fukuda, Hideaki Horikawa, Masami Yoshioka, Yasuko Ishikawa. (oral)

- "Development of artificial saliva using whey", The 57th Annual Meeting of Japanese Association for Oral Biology, Satellite Symposia. Niigata. September 11, 2015. Ishikawa Y, Pieczonka T, Bragiel A, Yabuuchi S, Takeuchi Y, Yanagisawa S, Fukuta K, Kim J, Kim D, Yoshioka M. (oral)

- "Muscarinic receptor-induced trafficking of AQP5 to nuclei and its role in rat parotid glands", The 57th Annual Meeting of Japanese Association for Oral Biology. Niigata. September 13, 2015. Bragiel Aneta, Pieczonka Tomasz and Yasuko Ishikawa. (poster)

4. Journal publication:

Title, journal, volume, number, paragraph, date, co-author.

- "Long-term administration of whey alters atrophy, gene expression profiles and dysfunction of salivary glands in elderly rats", Journal of Functional F-oods, Volume 21, March 2016, pages 349-358; Tomasz D Pieczonka, Aneta M Bragiel, Hideaki Horikawa, Kana Fukuta, Masami Yoshioka, Yasuko Ishikawa.