

## PROCEEDING

# Down-regulation of submandibular gland AQP5 following parasympathetic denervation in rats

Ahmad Azlina<sup>1</sup>, Xuefei Li<sup>3</sup>, Purevjav Javkhlan<sup>1,2</sup>, Takahiro Hasegawa<sup>1</sup>,  
Chenjuan Yao<sup>1</sup>, Tetsuya Akamatsu<sup>1</sup>, and Kazuo Hosoi<sup>1</sup>

<sup>1</sup>Department of Molecular Oral Physiology, <sup>2</sup>Department of Periodontology and Endodontology, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan ; and <sup>3</sup>International Division, Otsuka Pharmaceutical Factory, Inc., Naruto, Japan

**Abstract :** Following chorda tympani denervation (CTD, parasympathetomy), the protein levels of aquaporin5 (AQP5) as well as AQP1 and Na<sup>+</sup>K<sup>+</sup>ATPase  $\alpha$ -subunit in the rat submandibular gland (SMG) were found to be decreased significantly. However, the level of another membrane protein, dipeptidyl peptidase IV was not affected by CTD, suggesting a selective reduction of AQP5, AQP1, and Na<sup>+</sup>K<sup>+</sup>ATPase  $\alpha$ -subunit proteins by CTD. However, the AQP5 mRNA level was scarcely affected by CTD, which suggested that transcription process of AQP5 was unaffected by this operation. AQP5 protein was shown to be degraded *in vitro* by the extract of the SMG obtained from normal rat ; inhibitor experiments *in vitro* suggested cathepsin B was a responsible enzyme. Co-localization of AQP5 and LAMP-2, a lysosomal marker, implicated AQP5 is degraded in lysosomes. A significant increase in the protein levels of LC3-II, an autophagy marker, at day 1 after CTD, and co-localization of the LC3 protein and AQP5, suggested that CTD activated autophagy of SMG, leading to AQP5 degradation. *J. Med. Invest.* 56 Suppl. : 273-276, December, 2009

**Keywords :** chorda tympani denervation, AQP5, degradation, autophagy

## INTRODUCTION

Water channel protein family, AQPs, is consisted of a group of membrane proteins which facilitate water movement across the plasma membrane. There are 13 types of AQPs in mammalian tissues and they are distributed in almost all types of the tissue. A member of the family, AQP5 had been identified in various glandular tissues of exocrine type (1, 2) including the SMG (3). The SMG is controlled by parasympathetic nerves and innervated through chorda tympani. Previous report on

the effect of CTD showed that AQP5 protein levels in the rat SMG were significantly decreased (4). To clarify the cause of AQP5 down-regulation, we analyzed the possible mechanism in this study.

## METHODS

Seven-week-old male Sprague-Dawley rats, weighing 170-190 gram were purchased from SLC (Shizuoka, Japan). Some of them are known to express a mutant AQP5, which expression at the acinar cell membrane in the SMG is extremely reduced (5). Rats with normal AQP5 sequences were therefore selected for the present study. Chorda tympani denervation was performed under anesthesia with Nembutal (sodium pentobarbital, 50 mg/kg body weight) as described previously (6). All the

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Address correspondence and reprint requests to Kazuo Hosoi, Department of Molecular Oral Physiology, Institute of Health Biosciences, the University of Tokushima Graduate School, Kuramoto-cho, Tokushima 770-8504, Japan and Fax : +81-88-633-7324.

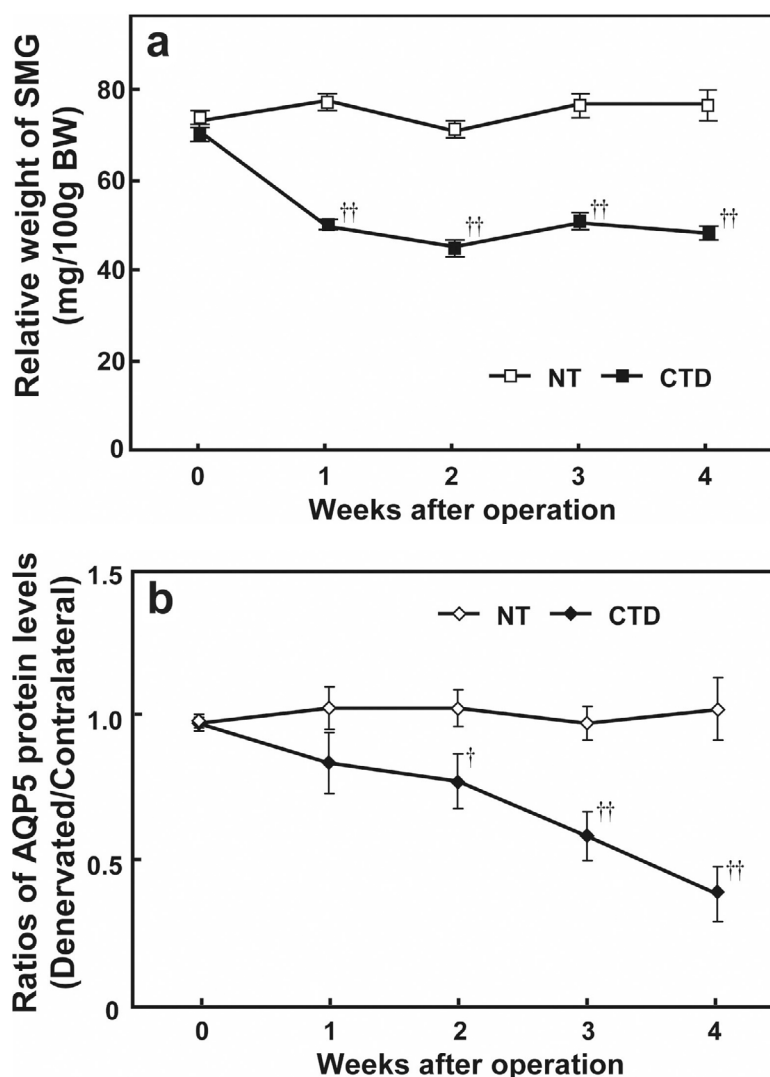
experimental protocol was approved by the Animal Ethical Committee of the University of Tokushima. The SMG tissues were processed for the assay for lysosomal enzymes activity, Western blotting, Northern blotting, and immunohistochemistry. Some of the untreated rats were sacrificed for preparation of the total membrane fraction and the SMG extract, which samples were subjected to the *in vitro* degradation assay of AQP5.

## RESULTS AND DISCUSSION

Effects of CTD on the SMG weight and the AQP5 expression level were examined. After CTD, the SMG weight was decreased but only for 1 week (Fig. 1a). The AQP5 level, on the other hand,

continuously decreased until week 4 (Fig. 1b). Western blotting of AQP1 and Na<sup>+</sup>K<sup>+</sup>ATPase  $\alpha$ -subunit showed a similar decrease. Administration of cevimeline hydrochloride (CM), an M3 muscarinic agonist, recovered the AQP5 and AQP1 protein levels reduced by CTD, but not the protein levels of Na<sup>+</sup>K<sup>+</sup>ATPase  $\alpha$ -subunit. Also, another membrane protein, dipeptidyl peptidase IV was scarcely affected by CTD and CM administration. These results suggested the selectivity of AQP5 protein reduction following CTD and/or effects of CM.

Northern blotting and real-time PCR experiments showed that the level of AQP5 mRNA was not affected by CTD suggesting that AQP5 mRNA transcription was not involved in the decrease of AQP5 protein levels. However, treatment of the CTD rats



**Figure 1** Effects of CTD on the weight and AQP5 protein levels of rat SMG. a. Relative weight of SMG between non-treated (NT) samples and denervated samples (CTD). b. Ratios of AQP5 protein levels of NT and CTD samples. Turkey's multiple analysis was then applied as a post hoc test. †  $P < 0.05$ , ††  $P < 0.01$ , significantly different from the NT group before the operation (0 wk). Modified from Li, *et al.* (4).

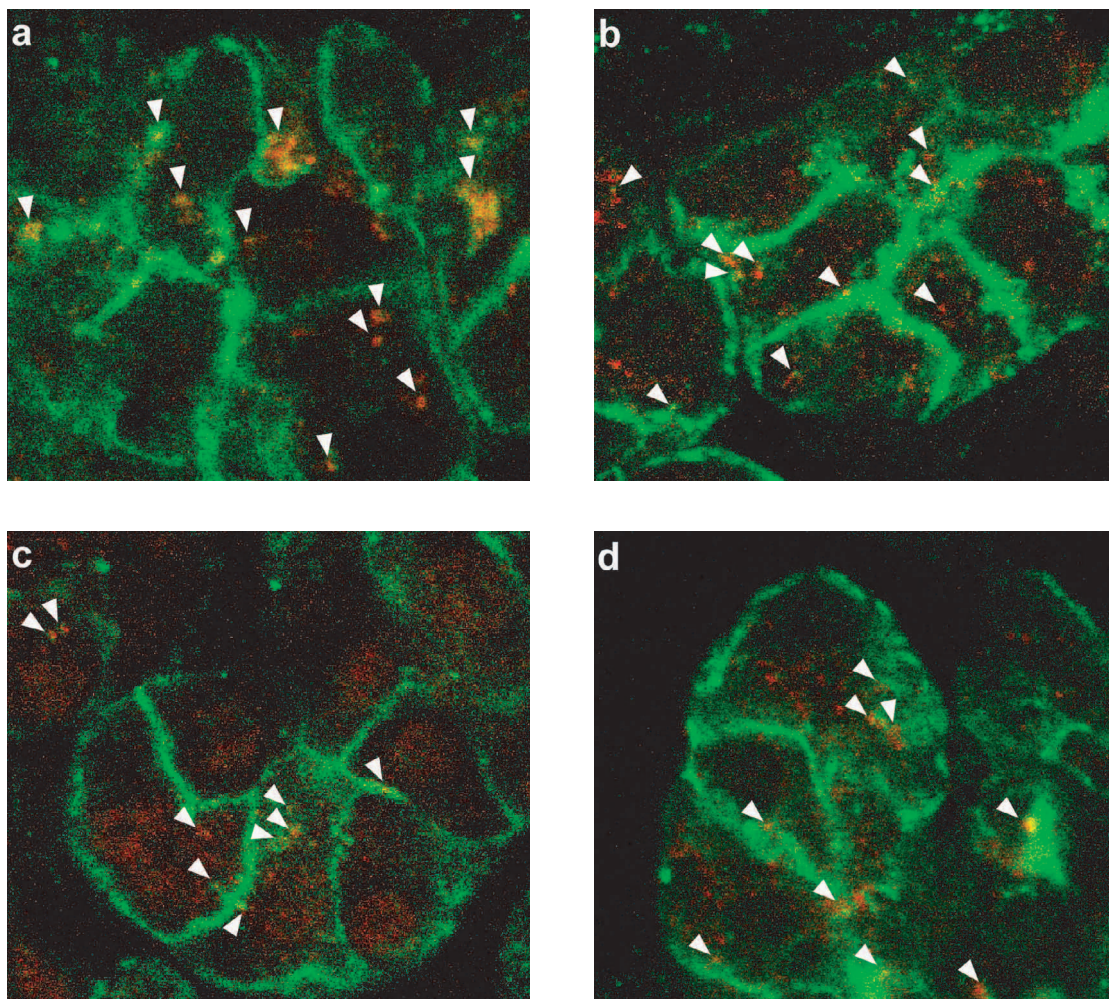
by chloroquine suppressed AQP5 reduction after the operation. Since chloroquine is a lysosomal denaturant, such results suggest involvement of the lysosomal degradation pathway in AQP5 reduction following CTD. The *in vitro* experiments showed that AQP5 can be degraded by the SMG extract which contains various proteolytic enzymes. The degradation experiment using inhibitors for aspartic proteases, serine/threonine proteases, and cysteine proteases showed that cathepsin B, one of cysteine proteases in the SMG extract, was responsible for AQP5 degradation. Since cathepsin B is a lysosomal enzyme, and since this result implied the involvement of lysosome, immunohistochemistry of AQP5 and LAMP-2, a lysosomes marker was conducted; the results showed that the two proteins were co-localized, suggesting that AQP5 is degraded in lysosomes by cathepsin B (Fig. 2a). However, the ratio of co-localized structure among the total AQP5

positive structures was not altered after CTD and remained same, implying that degradation by this pathway was not elevated by CTD (Fig. 2b, c, d).

By Western blotting, on the other hand, LC3-II, an autophagy marker (7), potentially increased at day 1 after CTD and gradually decreased thereafter which suggested that CTD activated autophagy. The number of LC3 positive structure was shown to be co-localized with AQP5 and significantly increased at day 1 after CTD, suggesting that autophagy is involved in AQP5 degradation.

### CONCLUSION

In acinar cells of the rat SMG, AQP5 was shown to be degraded in lysosomes by cathepsin B although this process was not elevated by CTD. CTD



**Figure 2** Confocal image of co-localization of AQP5 and LAMP-2, a lysosomal marker, in frozen section of the rat SMG tissue. a. Sample of non-treated rat. b. 1 day after CTD. c. 7 days after CTD. d. 14 days after CTD. Anti-AQP5 antibody of acinar cells was labeled with green fluorescence of 549 Alexa whereas LAMP-2, with punctate red fluorescence of 488 Alexa. The number of co-localization between the two antibodies was not elevated after CTD.

rather activated autophagy which was suggested to degrade AQP5 consequently.

## ACKNOWLEDGEMENTS

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