AQUAPORIN-1 AND -4 IN THE PERIODONTAL RUFFINI ENDINGS AND THE TRIGEMINAL GANGLION OF RATS

B.G.T.L. NANDASENA[1*], A. SUZUKI[2], Y. KAWANO[2], K.NOZAWA-INOUE[2], AND T. MAEDA[2]

[1]*Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya.*
[2]*Division of Oral Anatomy, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

Introduction

Aquaporins (AQPs) are a family of water-selective channels which provide a major pathway for osmotically driven water transport through cell membranes. Thirteen different members of this family show widespread tissue distribution. Six of them (AQP1, 3, 4, 5, 8, 9) have been reported in the central nervous system and express diverse functions including a bidirectional water transport between the brain and blood vessels, cerebrospinal fluid formation, neural signal transduction, osmoreception, and pathophysiological conditions (Lehmann et al., 2004).

The Ruffini endings are the primary mechanoreceptor in the periodontal ligament (Maeda et al., 1999). They are characterized by extensive arborizations of thick axon terminals and by an association with specialized Schwann cells called terminal Schwann cells. The cell membrane of the axon terminals and the terminal Schwann cell of the periodontal Ruffini endings showed caveolae, suggesting the existence of axon-Schwann cell interaction in the periodontal Ruffini endings and an active transport between axon terminals and terminal Schwann cells (Maeda et al., 1999). However, there is little information available regarding the molecules which are involved in transport between these except the report on Na^+-K^+-ATPase. Our preliminary study has shown AQP1 immunoreaction in the periodontal Ruffini endings (Nandasena et al., 2007).

The present study aimed to explore further the expression of AQP1 and 4 in the Ruffini endings in the rat upper incisors periodontal ligament and the ganglion of the trigeminal nerve which is its nerve of origin.

Materials and methods

All experiments in this study have been approved and performed based on the ethical guidelines of Niigata University Intramural Animal Use and Care Committee (approval number #161).

RNA was extracted from unfixed trigeminal ganglia and kidneys of 8-week-old three male Wistar rats. cDNA of AQP1 and 4 were produced by reverse transcript polymerization chain reaction (RT-PCR). An additional five Wistar rats were transcardially perfused with buffered 4% paraformaldehyde. The trigeminal ganglia and upper jaws were dissected and prepared for immunohistochemistry for AQP1 and AQP4. Double immunofluorescent labeling with AQP1 and either PGP 9.5 or S-100 was carried out in some sections.

Immunofluorescent labeled sections of the trigeminal ganglion for AQP1 were used for quantitative analysis. The cell number and cross-sectional areas of AQP1-positive neurons were calculated from 4 sections per animal using the NIH (National Institute of Health) image analysis (*http:rsb.info.nih.gov/ij/download.html*). Their frequency of size distribution was also measured. The number of trigeminal ganglion neurons was counted and frequency of AQP1 immunoreactivity was calculated.

Results

A reverse transcripted cDNA samples from the trigeminal ganglion and kidney showed AQP1 and 4 of expected sizes.

AQP1 immunoreactions were recognizable in the axon terminals of the periodontal Ruffini endings and their associated terminal Schwann cells. Immunoreactions of the axon terminals and the associated terminal Schwann cells for AQP1 were confirmed by a double staining with either S-100 or PGP 9.5

(Fig. 1a and b). No immunoreaction for AQP4 was detected in periodontal Ruffini endings.

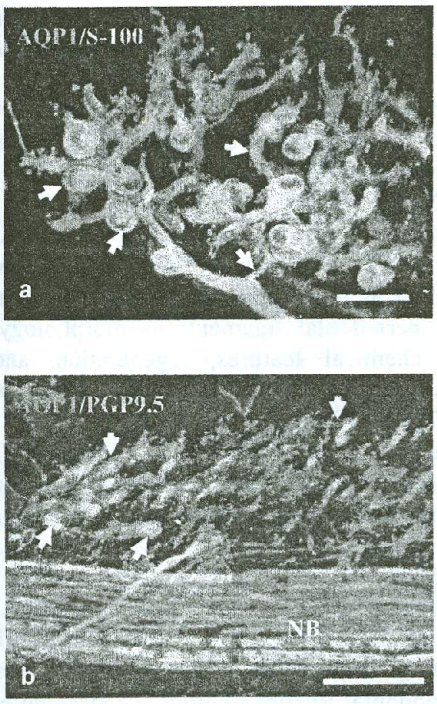

Figure 1. Double immunostaining of the periodontal ligament with AQP1 and either S-100 (a), or PGP 9.5 (b). Arrows in (a) indicate the co-localization of AQP1 with S-100 in the cell membrane of the terminal Schwann cells and their cytoplasmic extensions. Note the co-localization of AQP1 with PGP9.5 over the axon terminals (arrows in b). NB: nerve bundle. Scale bars: 25 μm in (a), 50 μm in (b).

Trigeminal neurons showed AQP1-positive immunoreactions, which were colocalized with PGP 9.5 (Fig. 2). AQP4 immunoreaction was localized in some satellite cells of the trigeminal ganglion.

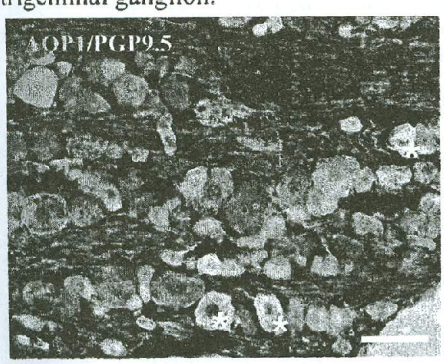

Figure 2. Immunoreaction for AQP1 in the rat trigeminal ganglion. Double immunostaining with AQP1 and PGP9.5 shows co-localization of AQP1 and PGP 9.5 immunoreaction in the trigeminal ganglion neurons (asterisks). Scale bars: 75 μm.

The frequency of AQP1 positive neurons to the total trigeminal ganglion neurons was 16.1%. The mean and median cross-sectional areas of them were 803.6 ± 336.9 μm² and 750 μm². 80% AQP1 positive neurons were above the cross-sectional area of 500 μm² (Fig. 03).

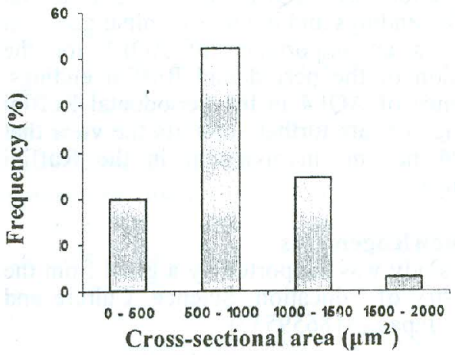

Figure 3. Size distribution of AQP1 positive neurons in the trigeminal ganglion.

Discussion

The present study shows for the first time AQP1 immunoreactions in the axon terminals of the periodontal Ruffini endings and in the trigeminal ganglion. This suggests a possible synthesis of AQP1 in trigeminal ganglion and conveying to the axon terminals.

The current quantitative analysis shows 16.1% of the trigeminal neurons to be positive for AQP1 while majority of them are categorized as medium-sized neurons. This correlates with the findings of Aita et al., (2006) that the medium-sized neurons mediate mechanotrans-duction and innervate the periodontal Ruffini endings.

The periodontal Ruffini endings lacked AQP4. However, some satellite cells of the trigeminal ganglion were positive for AQP4. To date, AQP4 has not been reported in Schwann cells under normal condition, although AQP4 in astroglia has been reported (Lehmann et al., 2004).

Since an active tissue remodeling take place in the periodontal ligament and as an active transport between axon terminals and terminal Schwann cell has been suggested by ultrastructural configurations (Maeda et al., 1999), we can suggest that the expression of AQP1 in axon terminals and terminal Schwann cells may involve in the maintenance and the water transport of the periodontal Ruffini endings. However, we cannot exclude the possibility that AQP1 in the periodontal Ruffini endings involves in neural signal transduction.

Conclusion

Expression of AQP1 in the periodontal Ruffini endings and in the trigeminal ganglion suggests an importance of AQP1 for the function of the periodontal Ruffini endings. Absence of AQP4 in the periodontal Ruffini endings of rats further supports the view that AQP4 has no involvement in the Ruffini endings.

Acknowledgements

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