View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Tokushima University Institutional Repository

PROCEEDING

Electrophysiological properties of AQP6 in mouse parotid acinar cells

Hideki Ichikawa[1,2], Yoshiyuki Shibukawa[1,2], Yoshinori Sahara[3], Maki Tsumura[1,2,4], Bing Qi[5], Keitaro Satoh[6], Takanori Narita[5], Sadamitsu Hashimoto[1,7], Yasunori Momose[4], Masakazu Tazaki[1,2], Masaki Shimono[7], and Hiroshi Sugiya[5]

[1]*Oral Health Science Center, hrc7 and* [2]*Department of Physiology, Tokyo Dental College, Chiba, Japan ;* [3]*Department of Physiology, Iwate Medical University School of Dentistry, Morioka, Japan ;* [4]*Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan ;* [5]*Department of Physiology, Nihon University School of Dentistry at Matsudo, Matsudo, Japan ;* [6]*Department of Regulatory Physiology, Dokkyo Medical University, School of Medicine, Tochigi, Japan ; and* [7]*Department of Pathology, Tokyo Dental College, Chiba, Japan*

Abstract : Salivary gland acinar cells secrete large amounts of water and electrolytes, where aquaporins (AQPs) are thought to be involved in the secretion. In the present study, we investigated expression/localization of AQP6, and the anion transporting properties of AQP6 in mouse parotid acinar cells. RT-PCR, western blotting and immunohistochemical analyses revealed expression of AQP6 in acinar cells, localized in apical membrane. Voltage ramp from -100 mV to +100 mV at a holding potential of -60 mV elicited outwardly-rectifying currents, in the presence of extracellular Cl^- channel blockers and intracellular solution with 150 mM Cs^+. These outward currents were increased when extracellular Cl^- was replaced by Br^-, NO_3^-, I^-, or SCN^-, accompanying a negative shift of reversal potentials. The outward current was enhanced by extracellular Hg^{2+}. These results were consistent with the biophysical properties of transfected AQP6 oocytes or HEK cells, which indicate that the AQP6 channel is functionally expressed in parotid acinar cells, and suggest that AQP6 contributes to secretion of anions in parotid acinar cells. J. Med. Invest. 56 Suppl. : 347-349, December, 2009

Keywords **:** *AQP6, parotid acinar cells, ion transport*

INTRODUCTION

Aquaporins (AQPs) are membrane proteins that facilitate water movement across the cell membrane. Salivary gland acinar cells secrete large amounts of water and electrolytes, and AQPs are thought to be participating in the former secretion. Expression of highly water-selective AQP5 has been demonstrated in salivary acinar cells, and reduction of saliva production and secretion of hypertonic, hypernatremic, and viscous saliva has been reported in AQP5 knock-out mice (1). On the other hand, AQP6 expression has also been reported in parotid acinar cells in rats (2). AQP6-transfected HEK cells are permeable not only to water, but also electrolytes. Therefore, in the present study, we investigated

Received for publication December 1, 2009 ; accepted December 8, 2009.

Address correspondence and reprint requests to Yoshiyuki Shibukawa, DDS, PhD, Department of Physiology, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba-city 261-8502, Japan and Fax : +81-43-270-3771.

the electrophysiological characteristics of AQP6 in mouse parotid acinar cells.

MATERIALS AND METHODS

All animals were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Council of the Japanese Pharmacological Society, the Physiological Society of Japan and the American Physiological Society. The study was approved by the Ethics and the Animal Committees of the Tokyo Dental College.

The parotid tissue was dissected from mice (C57Bl/6 : 12-20 weeks old), and enzymatically treated (collagenase/hyaluronidase at 37°C for 20 min) to separate single acinar cells. Standard extracellular solution contained (mM) : 136 NaCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 5 KCl, 10 HEPES, 10 glucose and 12 $NaHCO_3$, with pH 7.4 (290 mOsmol). Membrane currents were recorded with a whole-cell voltage-clamp configuration. Cells were arranged on the glass bottom of a recording chamber mounted on a microscope stage (Zeiss). Patch pipettes were pulled from capillary tubes (P80/PC, Sutter Instrument, Novato, CA) and filled with intracellular solution (Cs-ICS : Table 1). Whole-cell currents were recorded using a patch-clamp amplifier (L/M-EPC-7+, List-Medical). Data were filtered at 3 kHz and digitized at 10 kHz by using Digidata 1440 interface (Axon Instruments) and analyzed off-line using Axograph software (Axon Instruments). All recordings were made at room temperature (22-26°C).

For Western blotting, proteins in the membrane fraction of parotid glands were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and reacted with rabbit polyclonal anti-AQP6 antibody (Millipore).

For immunohistochemical analysis, the parotid gland was frozen in liquid nitrogen and sectioned. Immunofluorescence labeling was performed with rabbit polyclonal anti-AQP6 antibody, followed by Alexa-488 anti-rabbit IgG (Invitrogen).

For single acini RT-PCR, total RNA was isolated from isolated single parotid acinar cells for whole-cell recordings (Invitrogen). Reverse transcription (Qiagen) and cDNA amplification by PCR (Takara Bio) was performed. PCR products were purified (Sigma-Aldrich), and DNA fragments were separated by 2% agarose gel electrophoresis, followed by ethidium bromide staining.

For electron microscopic observation, fixed parotid glands were frozen. Silver-to-gold ultra-thin frozen sections were cut at -100°C (Reichert-Nissei), transferred onto a drop of 2.3 M sucrose, and mounted with Formvar-coated nickel grids. The samples floated on a drop of the anti-AQP6 polyclonal antibody were incubated, and transferred onto a drop of colloidal gold conjugated with anti-rabbit IgG. Cryosections were stained with uranyl acetate and lead citrate, then examined by transmission electron microscopy (Hitachi).

Table 1 Extracellular solution (pH 7.4 Tris)

(in mM)	NaCl	$NaNO_3$	NaI	NaBr	NaSCN	$CaCl_2$	$MgCl_2$	Hepes	DIDS	Niflumic acid
Cl-ECS	140	0	0	0	0	2.0	2.0	10	0.1	0.1
NO_3-ECS	0	140	0	0	0	2.0	2.0	10	0.1	0.1
I-ECS	0	0	140	0	0	2.0	2.0	10	0.1	0.1
Br-ECS	0	0	0	140	0	2.0	2.0	10	0.1	0.1
SCN-ECS	0	0	0	0	140	2.0	2.0	10	0.1	0.1

Intracellular solution (pH 7.2 by Tris)

(in mM)	CsCl	EGTA	MgATP	HEPES
Cs-ICS	135	5.0	2.0	10

RESULTS AND DISCUSSION

In freshly isolated acinar cells, a voltage ramp from -100 mV to +100 mV at a holding potential of -60 mV elicited outwardly-rectifying currents in the presence of extracellular 0.1 mM 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and 0.1 mM niflumic acid (with extracellular solution containing 140 mM Cl^- (Cl-ECS) and intracellular solution containing 150 mM Cs^+ (Cs-ICS) (Table 1)). In order to examine the anion permeability, NaCl of the extracellular fluid (Cl-ECS, Table 1) was substituted with an equimolar concentration of NaBr, $NaNO_3$, NaI, or NaSCN (Br-ECS, NO_3-ECS, I-ECS, or SCN-ECS, respectively ; Table 1). Outwardly-rectifying currents at a positive membrane potential showed an increase when Cl^- was replaced with these anions. The relative permeability of these anions were $SCN^- >> NO_3^- > I^- > Br^- > Cl^-$. The NO_3^- currents in the parotid acinar cells were enhanced by application of extracellular Hg^{2+}. RT-PCR and Western blot analyses revealed expression of AQP6 in parotid acinar cells. In addition, immunoreaction of AQP6 was strongly detected at apical membrane. In the present study, outward SCN^-, NO_3^-, I^-, Br^- and Cl^- currents showed no sensitivity to extracellular DIDS and niflumic acid and intracellular Cs^+. This indicates that Ca^{2+}-activates Cl^- channels such as transmembrane protein 16A (TMEM16A) (3) and that K^+ currents are not involved in these anion currents. However, Hg^+ sensitivity was consistent with that obtained from AQP6 expressed in oocytes or HEK cells (4). This indicates that AQP6 localized at apical membrane may be substantially involved in cellular permeability to anions, as well as water, in parotid acinar cells.

ACKNOWLEDGMENTS

This work was supported by HRC7, Grant-in-Aid (20592187) for Scientific Research from MEXT of Japan ; and a grant from the Dean of our school. We thank Professor Jeremy Williams for his assistance with the English of this manuscript.

REFERENCES

1. Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS : Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 274 : 20071-20074, 1999
2. Matsuki-Fukushima M, Hashimoto S, Shimono M, Satoh K, Fujita-Yoshigaki J, Sugiya H : Presence and localization of aquaporin-6 in rat parotid acinar cells. Cell Tissue Res 332 : 73-80, 2008
3. Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, Park SP, Lee J, Lee B, Kim BM, Raouf R, Shin YK, Oh U : TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 455 : 1210-1215, 2008
4. Ikeda M, Beitz E, Kozono D, Guggino WB, Agre P, Yasui M : Characterization of aquaporin-6 as a nitrate channel in mammalian cells. Requirement of pore-lining residue threonine 63. J Biol Chem 277 : 39873-39879, 2002