

**PROCEEDING****Electrophysiological properties of AQP6 in mouse parotid acinar cells**

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**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<sup>-</sup> channel blockers and intracellular solution with 150 mM Cs<sup>+</sup>. These outward currents were increased when extracellular Cl<sup>-</sup> was replaced by Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, I<sup>-</sup>, or SCN<sup>-</sup>, accompanying a negative shift of reversal potentials. The outward current was enhanced by extracellular Hg<sup>2+</sup>. 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

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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<sub>2</sub>, 0.5 MgCl<sub>2</sub>, 5 KCl, 10 HEPES, 10 glucose and 12 NaHCO<sub>3</sub>, 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	NaN <sub>3</sub>	NaI	NaBr	NaSCN	CaCl <sub>2</sub>	MgCl <sub>2</sub>	Hepes	DIDS	Nitrocellulose
Cl-ECS	140	0	0	0	0	2.0	2.0	10	0.1	0.1
NO <sub>3</sub> -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<sup>-</sup> (Cl-ECS) and intracellular solution containing 150 mM Cs<sup>+</sup> (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<sub>3</sub>, NaI, or NaSCN (Br-ECS, NO<sub>3</sub>-ECS, I-ECS, or SCN-ECS, respectively ; Table 1). Outwardly-rectifying currents at a positive membrane potential showed an increase when Cl<sup>-</sup> was replaced with these anions. The relative permeability of these anions were SCN<sup>-</sup> >> NO<sub>3</sub><sup>-</sup> > I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup>. The NO<sub>3</sub><sup>-</sup> currents in the parotid acinar cells were enhanced by application of extracellular Hg<sup>2+</sup>. 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<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, I<sup>-</sup>, Br<sup>-</sup> and Cl<sup>-</sup> currents showed no sensitivity to extracellular DIDS and niflumic acid and intracellular Cs<sup>+</sup>. This indicates that Ca<sup>2+</sup>-activates Cl<sup>-</sup> channels such as transmembrane protein 16A (TMEM16A) (3) and that K<sup>+</sup> currents are not involved in these anion currents. However, Hg<sup>2+</sup> 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.

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