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Immunolocalization of Water Channel Aquaporins in the Vomeronasal Organ of the Rat: Expression of AQP4 in Neuronal Sensory Cells

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Abstract

The vomeronasal organ comprises a pair of narrow tubes in the mammalian nasal septum, serving as a chemosensory system for pheromones. We examined the expression and localization of water channel aquaporins (AQPs) in the rat vomeronasal organ. AQP1 was localized in blood vessels, being particularly abundant in cavernous tissues of the nonsensory mucosa. AQP5 was found in the apical membrane of the gland acinar cells in the vomeronasal organ. AQP3 was detected in the basal cells of the nonsensory epithelium, whereas it was absent in the sensory epithelium. AQP4 was found in both the sensory and the nonsensory epithelia. Interestingly, AQP4 was highly concentrated in the sensory cells of the sensory epithelium. Immunoelectron microscopic examination clearly showed that AQP4 was localized at the plasma membrane in the cell body and lateral membrane of the dendrite, except for the microvillous apical membrane. Nerve fiber bundles emanating from neuronal sensory cells were positive for AQP4, whereby the plasma membrane of each axon was positive for AQP4. These observations clearly show that neuronal sensory cells in the vomeronasal organ are unique in that they express abundant AQP4 at their plasma membrane. This is in marked contrast to the olfactory and central nervous systems, where AQPs are not detectable in neurons, and instead, AQP4 is abundant in the supporting cells and astrocytes surrounding them. The present findings suggest a unique water-handling feature in neuronal sensory cells in the vomeronasal organ.

Key words: immunohistochemistry, nerve, pheromone, sensory cell, supporting cell, ultrastructure

Introduction

Aquaporins (AQPs) are integral membrane proteins that serve as channels in the transfer of water and small solutes such as glycerol and urea (Agre et al. 2002; Takata et al. 2004; Verkman 2005). At present, 13 isoforms of AQPs (AQP0–AQP12) have been identified and cloned in mammalian cells (Morishita et al. 2004). They are divided into 3 groups based on their sequence homology and functions: aquaporin (AQP), aquaglyceroporin, and superaquaporin subfamilies (Agre et al. 2002; Morishita et al. 2004). The AQP subfamily is composed of AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8 and is specifically involved in the transfer of water. The aquaglyceroporin subfamily is made up of AQP3, AQP7, AQP9, and AQP10 and is involved in the transfer of water as well as glycerol and urea. The superaquaporin subfamily comprises AQP11 and AQP12 and is more distantly related to other mammalian AQPs (Morishita et al. 2004; Ishibashi 2006).

Water transport via AQPs is important in maintaining homeostasis. They are highly and differentially expressed in water-handling organs including the kidney, exocrine glands, and blood vessels (Nielsen et al. 2002; Takata et al. 2004; Verkman 2005). Sensory organs such as the eye and inner ear are watery organs, where the transfer of water is critical in maintaining the sensing of external stimuli. In the eye, at least 5 isoforms of AQPs are expressed in a cell type–specific manner and play important roles in regulating the lens transparency, intraocular pressure, microenvironment of the retina, and tear secretion (Verkman 2003). In the ear, AQP1, AQP3, AQP4, AQP5, AQP7, and AQP8 are expressed, and they must work in concert

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in order to maintain fluid regulation in the inner ear (Huang et al. 2002).

In the olfactory system, we previously showed the distinct expression pattern of AQPs in the rat nasal mucosa (Ablimit et al. 2006). AQP3 and AQP4 are differentially expressed in the olfactory and respiratory epithelia. AQP3 is strongly expressed in the supporting cells of the olfactory epithelium and may serve in maintaining the specific microenvironment around sensory cells for olfaction (Ablimit et al. 2006).

The vomeronasal organ of animals was discovered by Ludvig Jacobson at the beginning of 19th century (Døving and Trotier 1998; Takami 2002). In many terrestrial tetrapods, a pair of vomeronasal organs are situated at the base of the nasal septum in the anterior nasal cavity (Døving and Trotier 1998; Takami 2002). The vomeronasal organ is part of the nasal chemosensory system, is distinct anatomically and physiologically from the olfactory system (Wysocki 1979; Halpern et al. 1998), and is regarded as a chemosensory organ for pheromones. Many animals use their vomeronasal organs to detect chemical cues released by congeners and in biological fluids (Døving and Trotier 1998). In mammals, vomeronasal organs seem to play a role in regulated social behavior and sexual preference, necessary for their existence and propagation.

Anatomically, the organ comprises a pair of tubes encapsulated in the vomer (Døving and Trotier 1998; Takami 2002). Posteriorly, the central lumen of the tube is blind ending, whereas the anterior opens into the nasopalatine or incisive ducts. The lumen is filled with fluid, and fluid handling seems to be closely related to the function of the vomeronasal organ (Takami 2002). The lumen is lined with 2 types of opposing epithelia: sensory epithelium and nonsensory epithelium. The sensory epithelium is located on the medial wall and the nonsensory epithelium on the opposite side.

The sensory epithelium is composed of a sensory cell layer and supporting cell layer. The supporting cell layer is located in the upper portion of the epithelium and is made up of supporting cells. The sensory cell layer is located in the middle to the base of the epithelium, where the cell bodies of neuronal sensory cells are packed. They extend dendrites upward, which penetrate the supporting cell layer and reach the surface of the epithelium for chemical reception. Supporting cells and dendrites of neuronal sensory cells form junctions between them. The sensory cells extend axons downward, which leave the epithelium, form bundles, and run along the nasal septa as the vomeronasal nerves. The vomeronasal nerves finally enter the cranial cavity and project to the accessory olfactory bulb (Takami 2002). The nonsensory epithelium is ciliated pseudostratified epithelium. A characteristic large blood vessel, cavernous tissue, and associated glands are found in the lamina propria of the nonsensory epithelium.

To shed light on the maintenance of the aqueous environment that is critical for the proper reception of external signals in the receptor cells in other sensory systems such as the eye, ear, and olfactory epithelium, we used AQP isoform–specific antibodies to determine their cellular and subcellular localization in the rat vomeronasal organ by immunofluorescence and immunoelectron microscopy. The results were compared with those from the olfactory mucosa. To our knowledge, this is the first report on the expression and localization of AQPs in the vomeronasal organ.

Materials and methods

Antibodies

Primary antibodies used were as follows: affinity-purified rabbit anti-AQP1, -AQP3, -AQP4, and -AQP5 antibodies (Matsuzaki et al. 1999a, 1999b, 2002); rabbit antibodies to AQP2, AQP6, AQP7, AQP9, AQP10, and AQP11 (Tajika et al. 2002; Takata et al. 2004; Morishita et al. 2005); and mouse anti-protein gene product 9.5 (PGP9.5) antibodies (Dennis et al. 2003). Secondary antibodies used were as follows: Rhodamine Red X-labeled donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA); fluorescein isothiocyanate–labeled donkey anti-mouse IgG (Jackson Immunoresearch); and gold particles conjugated to fragment antigen binding (Fab′) fragments of anti-rabbit IgG (Nanogold, Nanoprobes, Stony Brook, NY).

Animals and tissue preparation

Male Wistar rats, 4 weeks of age, were obtained from the Animal Breeding Facility, Gunma University Graduate School of Medicine (Maebashi, Japan). The protocol followed in this study was approved by the Animal Care and Experimentation Committee, Gunma University (07-099). Rats were anesthetized via an intraperitoneal injection of sodium pentobarbital (50 mg/0.1 ml) and killed by cervical dislocation. They were then decapitated, and the nasal portions containing the vomeronasal organ were removed together with the surrounding bone and fixed with 3% paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4 °C. They were then immersed in 20% sucrose in PBS overnight at 4 °C, embedded in optimal cutting temperature (OCT) compound (Sakura Finetechnical, Tokyo, Japan), rapidly frozen with liquid nitrogen, and stored at −80 °C. Cryostat sections 8- to 10-μm thick were cut with a Leica CM 1900 cryostat (Vienna, Austria) and mounted on silane-coated glass slides (Matsunami, Osaka, Japan). Sections were used for immunofluorescent labeling for light microscopic examination and immunogold labeling for electron microscopic examination.

Immunofluorescence microscopic examination

For immunofluorescent staining, sections were washed 3 times in PBS for 15 min and were then incubated with 5% normal donkey serum (NDS) in PBS for 15 min. Subsequently, sections were sequentially incubated with primary antibody solution for 2 h and fluorescently labeled secondary antibody solution for 1 h in a humidified chamber at room

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temperature. Both the primary and the secondary antibodies were diluted with 5% NDS. Double labeling was achieved by sequential incubation of a mixture of the primary antibodies raised in different animal species and a mixture of fluorescently labeled species-specific secondary antibodies against corresponding animal IgG. Nuclear staining was performed by adding either 4′,6 diamidino-2-phenylindole (DAPI) (Roche Diagnostics, Basel, Switzerland) or TO-PRO-3 (Molecular Probes, Carlsbad, CA) to the secondary antibody solution. After incubation with the antibodies, sections were washed thoroughly with PBS and mounted as described (Ablimit et al. 2006). They were examined with an Olympus BX-60 epifluorescence microscope equipped with Nomarski differential interference optics (Tokyo, Japan) or with an Olympus FV-1000 confocal laser microscope (Tokyo, Japan).

Immunoelectron microscopy

For immunoelectron microscopy, cryostat sections were washed 3 times in PBS for 15 min each and incubated with 5% normal goat serum (NGS) in PBS containing 0.02% saponin for 20 min. They were first incubated with the rabbit anti-AQP4 antibody (1:500 dilution in 5% NGS in PBS containing 0.005% saponin) overnight at 4 °C and washed with PBS containing 0.005% saponin 4 times for 5 min each. Next, the sections were incubated with gold particles conjugated to Fab′ fragments of anti-rabbit IgG diluted to 1:100 with 5% NGS in PBS containing 0.005% saponin for 2 h, and washed with PBS containing 0.005% saponin for 20 min with 6 changes. Sections were then fixed with 1% glutaraldehyde for 10 min, washed thoroughly with water, and incubated with silver enhancement solution comprising 1 mg/ml silver acetate, 14 mg/ml trisodium citrate dihydrate, 15 mg/ml citric acid monohydrate, and 2.5 mg/ml hydroquinone for 8 min (Sawada and Esaki 1994). After a quick rinse with water twice, they were immersed in 0.05% sodium acetate for 1 min and washed with water 6 times for 2 min each. The sections were treated with 0.05% gold chloride trihydrate for 2 min at room temperature, washed with water 6 times, and fixed with 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 7.4, trihydrate for 20 min (Shin et al. 1997). The specimens were washed with water 3 times, dehydrated with ethanol, and embedded in Epon. Ultrathin sections were cut, stained with uranyl acetate, and examined with a JEM-100CXII electron microscope (JEOL, Tokyo, Japan).

Results

The lumen of the vomeronasal organ is lined with 2 types of epithelia, that is, sensory epithelium and nonsensory epithelium. The sensory epithelium covers the medial wall, whereas the opposing nonsensory epithelium lies on the lateral wall. These epithelia are separated by a fluid-filled lumen. A large blood vessel, glands, and cavernous tissue are found in the lamina propria underneath the nonsensory epithelium (Figures 1A,D).

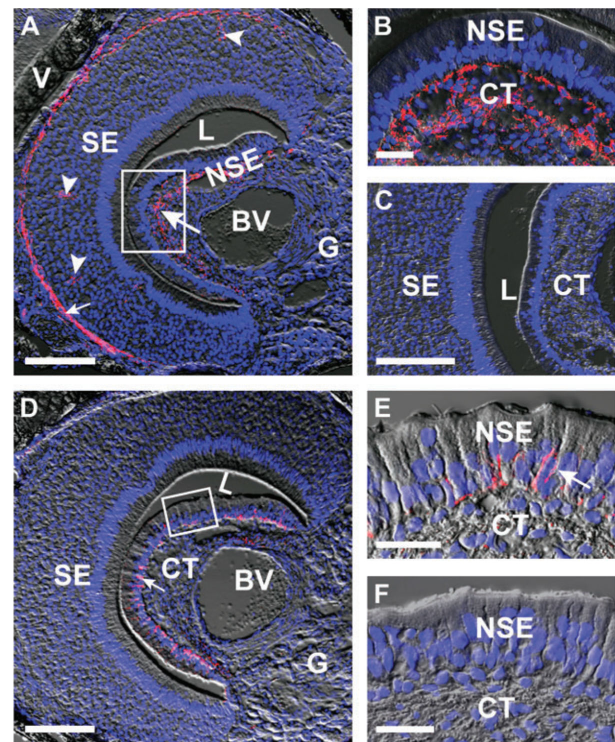

Figure 1 Immunofluorescence localization of AQP1 (A–C) and AQP3 (D–F) in the rat vomeronasal organ. AQP1 or AQP3 is shown in red. Nuclei were counterstained with DAPI (blue). SE, sensory epithelium; NSE, nonsensory epithelium; L, lumen; BV, blood vessel; V, vomer; CT, cavernous tissue; G, gland. Confocal fluorescence images are projected onto Nomarski images. Bars: 200 μm (A, C, and D) and 50 μm (B, E, and F). **(A–C)** AQP1. A survey view of the vomeronasal organ (A) shows that AQP1 (red) is expressed in the cavernous tissue on the nonsensory side (large arrow) and in the lamina propria on the sensory side (small arrow). The indentation of the lamina propria into the epithelium is also positive for AQP1 (arrowheads). The area indicated with a rectangle is enlarged in (B). Epithelial cells in either sensory or nonsensory epithelium are not positive for AQP1. Cells in the cavernous tissue are positive for AQP1 (B). When labeling for AQP1 was carried out in the presence of the antigen peptide, no positive labeling is detected (C). **(D–F)** AQP3. A survey view of the vomeronasal organ (D) shows that AQP3 (red) is expressed in the cells of the nonsensory epithelium (arrow). The area indicated with a rectangle is enlarged in (E). A small number of epithelial cells (arrow) in the nonsensory epithelium are weakly positive for AQP3 (red) (E). When labeling for AQP3 was carried out in the presence of the antigen peptide, no positive labeling is detected in the corresponding regions (F).

Previous immunoblotting experiments showed the expression of AQP1, AQP3, AQP4, and AQP5 in the nasal mucosa (Ablimit et al. 2006). Preliminary immunofluorescence staining for AQP1, AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP9, AQP10, and AQP11 (data not shown) revealed the presence of AQP1, AQP3, AQP4, and AQP5 in the vomeronasal organ. Therefore, we examined their localization in more detail in this study (data not shown).

AQP1 was found in the connective tissues in both the sensory and the nonsensory epithelial sides. AQP1 was not detected in either the sensory or the nonsensory epithelium. On the sensory side, AQP1 was expressed in endothelial cells

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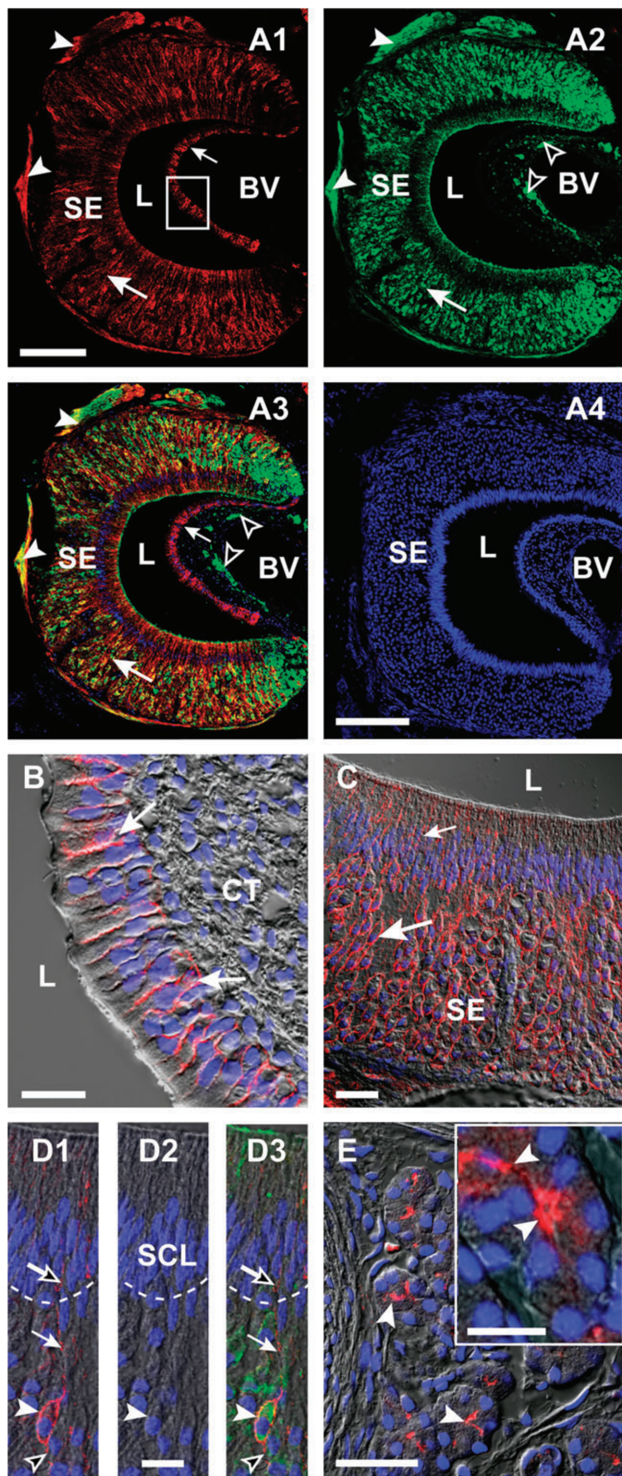

Figure 2 Immunofluorescence localization of AQP4 (A–D) and AQP5 (E) in the vomeronasal organ. AQP4 is shown in red. Nuclei were counterstained with DAPI (blue). SE, sensory epithelium; L, lumen. BV, blood vessel; CT, cavernous tissue. Bars: 200 μm (A), 50 μm (B, C, and E), and 20 μm (D and inset in E). **(A1–A3)** A survey view. AQP4 (red) is expressed in the cells of both sensory and nonsensory epithelia (large and small arrows, respectively, in A1 and A3). The area indicated with a rectangle is enlarged in (B). Double labeling with a neuronal marker, PGP9.5, shows the similar localization of AQP4 (red in A1 and A3) and PGP9.5 (green in A2 and A3) in the sensory epithelium. In the underlying lamina propria, AQP4 is also strongly expressed in nerve fiber bundles in the sensory mucosa (white arrowheads) but not in the nonsensory mucosa (black arrowheads). A3 is a merged image of AQP4 (A1) and PGP9.5 (A2). **(A4)** When labeling for AQP4 was carried out in the presence of the antigen peptide, no positive labeling is detected in the corresponding regions. **(B)** AQP4 is expressed in the nonsensory epithelium (arrows). **(C)** AQP4 is strongly expressed in the sensory epithelium, especially in the sensory cell layer (large arrow) and dendrites penetrating the supporting cell layer (small arrow). **(D)** In the sensory epithelium, AQP4 (red) is expressed in the neuronal sensory cell body (white arrowhead), its dendrite (black and white arrows), and axon (black arrowheads). The dotted lines show the lower border of the supporting cell layer (SCL). Note that AQP4-positive dendrites penetrate SCL (black arrows). The section was double stained for AQP4 (D1 and D3, red) and PGP9.5 (D3, green). D3 is a merged image of AQP4 and PGP9.5. **(E)** AQP5 (red) is present in the acinar cells (arrowheads). Inset shows the enlargement of acinar cells where AQP5 is localized on their apical side (arrowheads).

of blood vessels that are abundant just beneath the sensory epithelium. Surrounding connective tissue cells were also positive for AQP1 (Figure 1A). Neuronal sensory cells and nonsensory supporting cells in the sensory epithelium were negative for AQP1. On the nonsensory epithelial side, AQP1 was noted as abundant in the cavernous tissue situated between the nonsensory epithelium and a large blood vessel (Figure 1A,B). The large blood vessel itself was negative for AQP1.

AQP3 was not detected on the sensory epithelial side in either the epithelium or the lamina propria underneath. Instead, AQP3 was found in the epithelial cells of the nonsensory epithelial side (Figure 1D). Weak immunoreactivity for AQP3 was restricted to the basal cells (Figure 1E, arrow). The other epithelial cells were negative for AQP3.

AQP4 was abundantly expressed in the vomeronasal organ. It was detected in both the sensory and the nonsensory epithelia (Figure 2A). In order to identify neuronal sensory cells and nerve fibers, double staining was performed with a neuronal cell marker, PGP9.5 (green in Figure 2A). On the sensory side, sensory epithelium and nerve fiber bundles were positively stained for AQP4. The sensory epithelium is made up of the sensory cell and supporting cell layers (Figure 2C) (Takami 2002). AQP4 was found in both the sensory and the supporting cell layers (Figure 2A,C,D). Detailed examination of the epithelium was carried out by double labeling for AQP4 and PGP9.5. In the sensory cell layer, AQP4 was present in the cell body of neuronal sensory cells. Axons and dendrites extending from them were also positive for AQP4 (Figure 2C). In the supporting cell layer, AQP4 was not detected in the supporting cells themselves (Figure 2C,D). Instead, it was localized in the dendrites, the apical extension of the neuronal sensory cells, except for their tips facing the lumen. Nerve fiber bundles running close to the sensory epithelium were also strongly stained for AQP4 (Figure 2A).

On the nonsensory side, AQP4 was restricted to the epithelial cells. AQP4 was strongly positive in the epithelial cells (Figure 2B). AQP4 was localized along the basolateral

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membrane. The apical membrane was negative for AQP4. Nerve fiber bundles, which were positively labeled for PGP9.5, were negative for AQP4 on the nonsensory side (Figure 2A).

AQP5 was present in the vomeronasal gland, where it was localized at the apical membrane of secretory acinar cells (Figure 2E). AQP1, AQP3, or AQP4 were not found in the gland. AQP5 was not detected in either the sensory or the nonsensory epithelium.

The immunofluorescent labeling described above was completely abolished by incubation with primary antibodies in the presence of the antigen peptides used to raise the antibodies (e.g., Figures 1C,F and 2A4)

To further explore the localization of AQP4 in the sensory epithelium, immunoelectron microscopic examination was carried out by the pre-embedding method using the Nanogold probe. The dendrites, or the extension of sensory cells, have clear cytoplasm and are juxtaposed to the apical portions of the supporting cells with darker cytoplasm in the upper part of the epithelium (Figure 3A). AQP4 labeling was localized to the plasma membrane of dendrites (Figure 3B). The opposing plasma membrane of supporting cells was not labeled. The apical membrane with numerous microvilli was negative for AQP4 in both the sensory and the supporting cells (Figure 3B). In the middle part of the sensory epithelium, AQP4 was also detected along the plasma membrane of the cell body of neuronal sensory cells (Figure 4A,B). In the nerve fiber bundles running just beneath the epithelium, AQP4 was found along the plasma membrane of axons (Figure 4C). Ensheathing cells surrounding these axons were not labeled. The positive labeling for AQP4, as described above, was specific because it was abolished in the presence of antigen peptide (data not shown). These results clearly show that the expression of AQP4 is restricted to the sensory cells, where it is localized along the plasma membrane except for the apical membrane that faces the lumen. The localization of AQP4 in the sensory epithelium is schematically illustrated in Figure 5. The expression of AQPs in the sensory and nonsensory mucosa of the vomeronasal organ is summarized in Table 1.

Discussion

In this study, we identified the expression and localization of AQP1, AQP3, AQP4, and AQP5 in the vomeronasal organ. Among them, AQP4 was expressed in the neuronal sensory cells. To our knowledge, this is the first report that AQP4 is expressed at the plasma membrane of nerve cells.

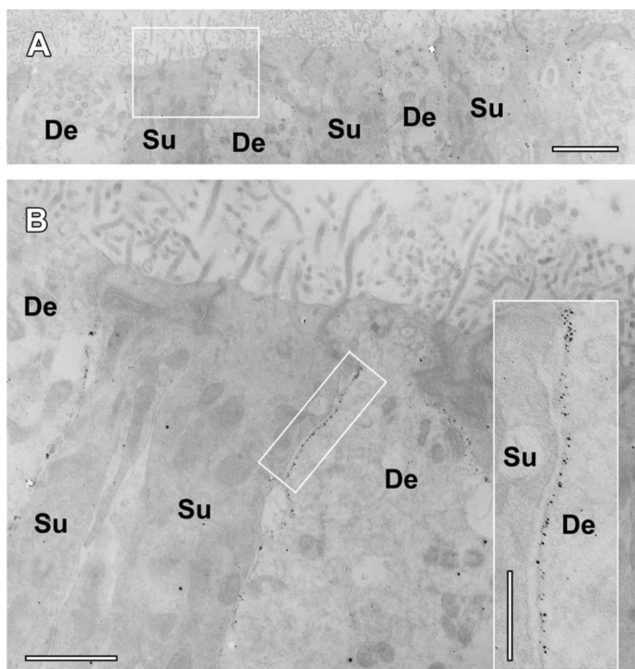

Figure 3 Ultrastructural localization of AQP4 in the sensory epithelium of the vomeronasal organ. De, dendrite of the neuronal sensory cell; Su, supporting cell. Bars: 2 μm (A), 1 μm (B), and 0.5 μm (inset). **(A)** A survey view of the surface of the sensory epithelium. The area indicated with a rectangle is enlarged in (B). **(B)** Gold labeling representing AQP4 is seen along the lateral border of the cells. Inset shows the enlargement of the area indicated with a rectangle. Note that gold labeling is restricted to the plasma membrane of the dendrite of neuronal sensory cells (De).

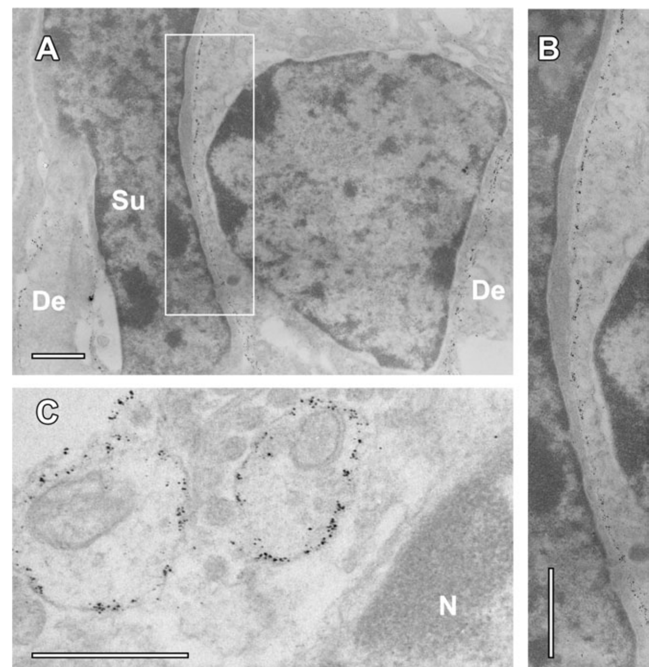

Figure 4 Ultrastructural localization of AQP4 in the vomeronasal organ. De, dendrite of the neuronal sensory cell; Su, supporting cell. Bars: 1 μm (A), 1 μm (B), and 0.5 μm (C). **(A)** Middle portion of the sensory epithelium. Gold labeling for AQP4 is seen along the plasma membrane. The area indicated with a rectangle is enlarged in (B). **(B)** Gold labeling representing AQP4 is seen along the plasma membrane of the cell body of the neuronal sensory cell. **(C)** In the nerve fiber bundles in the lamina propria underneath the sensory epithelium, AQP4 is localized along the plasma membrane of axons. N, nucleus of an ensheathing cell.

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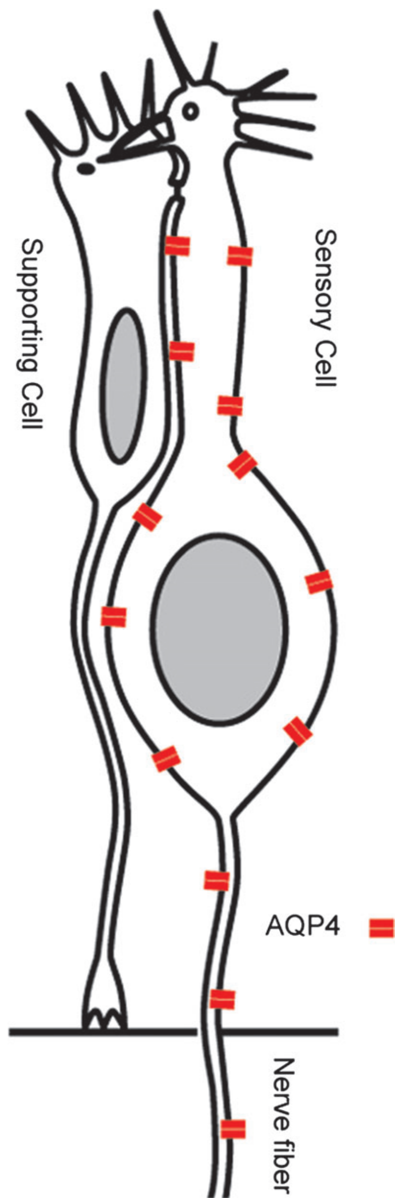

Figure 5 Schema showing the localization of AQP4 in the vomeronasal organ. Note that AQP4 is expressed in the neuronal sensory cells, where it is localized along the plasma membrane except for the apical membrane.

AQP4 is mainly expressed in the kidney, eye, and nervous tissues. In the central nervous system, AQP4 is strongly expressed in astroglial cells (Nielsen et al. 1997). It is concentrated in the glia limitans and endfeet located at the interface of the blood–brain barrier. It is also present in the ependyma and pial surfaces in contact with cerebrospinal fluid (Verkman 2005). In the eye, AQP4 is expressed in the retinal Müller cells that support bipolar cells (Hamann et al. 1998). In the rat cochlea, AQP4 is localized in supporting epithelial cells including Hensen's and inner ear sulcus cells (Mhatre et al. 2002). In the nasal olfactory mucosa, AQP4 is expressed in the supporting and basal cells but not in the neuronal sensory cells (Ablimit et al. 2006). All of these observations show that, in the nervous tissues, AQP4 is localized not in nerve cells per se but in supporting cells that are situated in close contact with nerve cells. In this context, AQP4 serves in maintaining the aqueous environment around nerve cells, thereby contributing to the functioning of nerve cells.

The sensory epithelia of both the olfactory mucosa and the vomeronasal organ comprise a sensory system for chemical stimuli located in the nasal cavity. They have a similar structure in that neuronal sensory cells are embedded in the sensory epithelium and are surrounded by supporting cells. Surprisingly, the localization of AQPs is quite different between them. In the olfactory mucosa, AQP4 is localized in the supporting cells (Ablimit et al. 2006); therefore, it seems to be involved in water handling around neuronal sensory cells (Huang et al. 2002; Takata et al. 2004) and serve in maintaining the milieu for olfaction (Verkman 2005). In the vomeronasal organ, on the other hand, AQP4 is concentrated in the neuronal sensory cells themselves. These results suggest that AQP4 in the sensory cells is directly involved in the handling of water around them. Electron microscopic examination showed that the vomeronasal organ is morphologically mature by 3 weeks of age (Garrosa and Coca 1991). It is of interest to examine the expression level of AQP4 when the rats become sexually mature.

Marked variation is also seen in the expression of AQP3. AQP3 was not detected in neuronal sensory cells, supporting cells, or basal cells. In the olfactory epithelium, AQP3 is absent in the neuronal sensory cells but is abundant in the supporting cells (Ablimit et al. 2006). It is not clear why the expression of AQP3 and AQP4 is different between the sensory epithelia of the vomeronasal organ and that of the olfactory mucosa. The difference may be attributed to the variation in the environment of these two organs: the olfactory mucosa is directly facing the empty nasal cavity, whereas the lumen of the vomeronasal mucosa is always filled with liquid.

AQP4 was found in the nerve fibers emanating from the sensory epithelium. These fibers are composed of axons of the neuronal sensory cells and ensheathing cells surrounding them. The present immunoelectron microscopic examination revealed that AQP4 is present along the plasma membrane of axons. This observation reveals that AQP4 is present along the entire plasma membrane except for the apical membrane in the neuronal sensory cells. Nerve fiber bundles running in the lamina propria of the nonsensory mucosa were negative for AQP4, a marked contrast to the presence of AQP4 in the axon forming the vomeronasal nerve, suggesting the distinct feature of water handling in the neuronal sensory cells of the vomeronasal organ.

In the nonsensory epithelium of the vomeronasal organ, AQP3 and AQP4 were expressed, which is also the case in the ordinary respiratory epithelium (Matsuzaki et al. 1999b; Ablimit et al. 2006), suggesting that the water-handling

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Table 1 Expression of AQPs in the vomeronasal organ

		AQP1	AQP3	AQP4	AQP5
Sensory mucosa	Sensory cells	– (–)	– (–)	+ (–)	– (–)
	Supporting cells	– (–)	– (+)	– (+)	– (–)
	Nerve fibers	– (–)	– (–)	+ (–)	– (–)
	Blood vessels	+ (+)	– (–)	– (–)	– (–)
Nonsensory mucosa	Columnar cells	– [–]	– [–]	+ [+]	– [–]
	Basal cells	– [–]	+ [+]	+ [+]	– [–]
	Cavernous tissue cells	+	–	–	–
	Glands	– [–]	– [–]	– [–]	+ [+]
	Nerve fibers	– [–]	– [–]	– [–]	– [–]

For comparison, expression of AQPs in the olfactory mucosa is shown in parentheses. In addition, expression of AQPs in the respiratory mucosa is shown in brackets. +, present; –, absent.

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machinery in the nonsensory epithelium is similar to that in the respiratory epithelium.

AQP5 was expressed at the apical membrane in the exocrine glands of the vomeronasal organ. Expression of AQP5 at the apical membrane is also found in Bowman's gland in the olfactory mucosa (Ablimit et al. 2006) and is a common feature in exocrine glands including the salivary glands, lacrimal gland, and duodenal gland (Matsuzaki et al. 1999a, 2004). At the basolateral membrane, AQP3 and AQP4 are present in the Bowman's gland (Ablimit et al. 2006), but neither of them was found in other glands including the vomeronasal organ. This observation suggests that the function of glands in the vomeronasal organ may not be as specific as that of Bowman's gland in the olfactory mucosa, whose secretion clears the surface of the sensory cells in the olfactory mucosa to facilitate olfaction.

AQP1 was found in the endothelial cells of small blood vessels. It was also noted in the surrounding connective tissue cells including cavernous tissue cells in the vomeronasal organ. Expression of AQP1 in the blood vessels and connective tissue cells is common in other tissues including the olfactory mucosa (Takata et al. 2004; Ablimit at al. 2006) and may be involved in water transfer to and/or from the blood.

In summary, we showed that the localization of AQPs in the vomeronasal organ is distinct from that in the olfactory mucosa. AQP4 was specifically expressed in the neuronal sensory cells, which is also a unique feature among neuronal cells.

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