V-ATPase expression in the mouse olfactory epithelium

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Păunescu TG, Jones AC, Tyszkowski R, Brown D. V-ATPase expression in the mouse olfactory epithelium. Am J Physiol Cell Physiol 295: C923–C930, 2008.—The vacuolar proton-pumping ATPase (V-ATPase) is responsible for the acidification of intracellular organelles and for the pH regulation of extracellular compartments. Because of the potential role of the latter process in olfaction, we examined the expression of V-ATPase in mouse olfactory epithelial (OE) cells. We report that V-ATPase is present in this epithelium, where we detected subunits ATP6V1A (the 70-kDa “A” subunit) and ATP6V1E1 (the ubiquitous 31-kDa “E” subunit isoform) in epithelial cells, nerve fiber cells, and Bowman’s glands by immunocytochemistry. We also located both isoforms of the 56-kDa B subunit, ATP6V1B1 (“B1,” typically expressed in epithelia specialized in regulated transepithelial proton transport) and ATP6V1B2 (“B2”) in the OE. B1 localizes to the microvilli of the apical plasma membrane of sustentacular cells and to the lateral membrane in a subset of olfactory sensory cells, which also express carbonic anhydrase type IV, whereas B2 expression is stronger in the subapical domain of sustentacular cells. V-ATPase expression in mouse OE was further confirmed by immunoblotting. These findings suggest that V-ATPase may be involved in proton secretion in the OE and, as such, may be important for the pH homeostasis of the neuroepithelial mucous layer and/or for signal transduction in CO₂ detection.

Numerous studies have reported the expression of carbonic anhydrase (CA) in specific cells of the mammalian olfactory epithelium (OE) (6, 10, 11, 29). The first report describing CA in OE cells used an enzymatic activity assay that did not allow the identification of a specific CA isoform (6). CA type II (CA II) was assumed to be the main CA isoenzyme expressed in this tissue, and this was subsequently established by immunohistochemical studies (19, 40). The genes for 10 different CA isoforms have been detected by RT-PCR in the human olfactory mucosa, and two of the isoenzymes, CA II and CA type IV (CA IV), were also described by immunohistochemistry (40). CA-catalyzed production of protons and bicarbonate ions was hypothesized to mediate the opening of cGMP-sensitive cyclic nucleotide-gated channels in a subpopulation of olfactory sensory neurons (19).

The mechanisms through which protons resulting from the reversible hydration of CO₂ catalyzed by CA II and other cytosolic CA isoforms are transported across the OE cell plasma membrane are unknown. Moreover, the OE is functionally involved in CO₂ detection. As such, this tissue could be implicated in acid-base sensing and/or regulation (40), implying that a number of unreported acid-base transporters may be present in the olfactory mucosa. Additionally, proton-secreting cells in various epithelial tissues express high levels of CA and, also, vacuolar proton-pumping ATPase [vacuolar, or V-type, H⁺-ATPase (V-ATPase)]. However, to our knowledge, there is no report in the literature addressing whether V-ATPase is expressed in the OE. Consequently, we are prompted to investigate this possibility in the present study.

We recently raised specific antibodies against a number of V-ATPase subunits and subunit isoforms (4, 20, 32) that have allowed us to pursue this question.

V-ATPase mediates the acidification of various intracellular organelles, including endosomes, lysosomes, the trans-Golgi network, and synaptic vesicles. This enzyme is also highly expressed in the plasma membrane domain of cells specialized for active proton transport into, and the pH regulation of, extracellular compartments. V-ATPase is a large (900-kDa) complex enzyme containing at least 13 distinct subunits, some of which occur in mammalian tissues as multiple isoforms (5). The 56-kDa “B” subunit is expressed as two highly homologous isoforms: ATP6V1B1 (“B1”) is present at high levels in specialized proton-secreting cells in a restricted number of organs and tissues, including the urogenital system, inner ear, eye, and lung; the quasi-ubiquitous ATP6V1B2 (“B2”) isoform is expressed in most cell types, where it plays a major role in organellar acidification (41).

Our present results show that V-ATPase is expressed in the mouse OE. Various subunits of this enzyme, including the V-ATPase 56-kDa B1 subunit isoform, were detected in certain cells of the olfactory mucosa. These findings suggest that V-ATPase is involved in proton secretion in the OE and, as such, may be important for the pH homeostasis of the neuroepithelial mucous layer and/or for signal transduction in CO₂ detection.

MATERIALS AND METHODS

Antibodies. To reveal V-ATPase expression in the mouse OE, we used previously characterized affinity-purified polyclonal antibodies raised in rabbit against mouse ATP6V1A (the 70-kDa “A” subunit) (20) and ATP6V1B2 (the 56-kDa “B2” subunit isoform) (32) and in chicken against ATP6V1E1 (the ubiquitous 31-kDa “E1” subunit isoform) (4, 18). A polyclonal rabbit anti-B1 V-ATPase antibody was raised against a peptide corresponding to the 13 COOH-terminal amino acids of the mouse ATP6V1B1 sequence coupled to keyhole limpet hemocyanin via a cysteine residue. The peptide was synthesized by the Massachusetts General Hospital Peptide/Protein Core Facility, and two rabbits were immunized by Cocalico Biologicals (Reamstown, PA). Immune sera were affinity purified against the

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imunizing peptide using the SulfoLink kit (Pierce, Rockford, IL) according to the manufacturer’s specifications.

The monoclonal anti-tubulin antibody produced in mouse was purchased from Sigma-Aldrich (St. Louis, MO), the sheep anti-human CA II antibody from Abcam (Cambridge, MA), and the goat anti-mouse CA IV antibody from R & D Systems (Minneapolis, MN). Polyclonal rabbit antibodies against olfactory marker protein (Sigma-Alrich) and protein gene product 9.5 (Dako, Carpinteria, CA) were used as olfactory markers.

The secondary antibodies were indocarbocyanine (Cy3)- or FITC-conjugated goat anti-rabbit, Cy3-conjugated donkey anti-chicken or anti-goat and mouse anti-rabbit, and FITC-conjugated donkey anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA) antibodies and Alexa Fluor 488 anti-sheep and anti-mouse or Alexa Fluor 594 anti-rabbit antibodies raised in donkey (Invitrogen, Carlsbad, CA).

Tissue preparation and immunohistochemistry. Wild-type (Atp6v1b1−/−; C57BL6, Jackson Laboratory, Bar Harbor, ME) and B1 V-ATPase-deficient (Atp6v1b1−/−) (15) adult mice or mouse pups (6 days old) were deeply anesthetized with a lethal dose of pentobarbital sodium (Nembutal, Abbott Laboratories, Abbott Park, IL; 200 mg/kg body wt ip), and the head was dissected and fixed with 4% paraformaldehyde in PBS for 4 h at room temperature and subsequently overnight at 4°C. Four pups (2 males and 2 females) and 15 adult mice (10 males and 5 females) were used in this study. All animal studies were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care in accordance with the National Institutes of Health, Department of Agriculture, and American Association for the Accreditation of Laboratory Animal Care requirements.

After extensive PBS washes, fixed tissues were stored at 4°C in PBS containing 0.02% sodium azide until use or decalcified using Immunocal (Decal Chemical, Tallman, NY) or Cal-Ex (Fisher Scientific, Pittsburgh, PA), air-dried, and stored at 4°C. Sections were rehydrated in PBS, treated with SDS [1% (wt/vol) in PBS for 4 min (7)], washed in PBS, and incubated with 1% bovine serum albumin for 15 min and then with the primary antibody for 90 min at room temperature, as previously described (33, 34). Slides were washed again, incubated for 1 h with the respective secondary antibody, and then rinsed and mounted in Vectashield medium (Vector Laboratories, Burlingame, CA) containing 4,6-diamidino-2-phenylindole (DAPI) where indicated. For dual immunostaining, the two primary antibodies were applied simultaneously, and then the secondary antibody mixture was applied.

Digital images were acquired using an epifluorescence microscope (Eclipse 800, Nikon Instruments, Melville, NY) outfitted with a charge-coupled device camera (Orca 100, Hamamatsu, Bridgewater, NJ), as described previously (32), and analyzed using IPLab version 3.2.4 image-processing software (Scanalytics, Fairfax, VA).

For confocal laser scanning and spinning disk confocal microscopy, tissue sections were prepared in a similar manner. Confocal imaging was performed on a confocal microscopy system (Radiance 2000, Carl Zeiss MicroImaging, Thornwood, NY) using LaserSharp 2000 version 6.0 software. For spinning disk confocal microscopy, images were collected using an inverted microscope (Eclipse TE2000-U, Nikon Instruments) equipped with a spinning disk confocal unit (UltraVIEW, Perkin-Elmer, Waltham, MA) and a digital camera (C9100 EM-CCD, Hamamatsu). For epifluorescence and confocal microscopy, images were subsequently imported into Photoshop version 6.0 image-editing software (Adobe Systems, San Jose, CA).

Immunoblotting. OE and kidneys from adult mice anesthetized as described above were dissected and homogenized in buffer containing 10 mM Tris·HCl (pH 7.4), 160 mM NaCl, 1 mM EGTA, 1 mM EDTA. Complete protease inhibitors (Roche Applied Science, Indianapolis, IN), 1% Triton X-100, and 0.05% Igepal CA-630. Homogenates were centrifuged for 15 min at 16,200 g at 4°C. SDS-PAGE and Western blotting were performed as previously described (32, 34) using the rabbit anti-B1 V-ATPase polyclonal antibody described above as whole serum and a commercial horseradish peroxidase conjugated mouse anti-rabbit antibody (Sigma-Aldrich).

RESULTS

Detection of V-ATPase in mouse OE. Immunofluorescence was used to investigate the expression and localization of various V-ATPase subunits in the OE of adult mice and 6-day-old mouse pups. Cryosectioning of the pup nasal cavity did not necessitate prior decalcification, which was only performed in this case as a control. Adult tissues were sectioned under decalcified and nondecalcified conditions. Decalcified tissues exhibited better morphology, but tissue antigenicity was better preserved when decalcification was not performed, entailing the use of pup and adult tissues for a complete study. Immunostaining results were qualitatively similar in adult and young animals, whether the tissues were decalcified or not, unless otherwise specified.

ATP6V1A (the V-ATPase 70-kDa “A” subunit) was strongly expressed in OE cells (Fig. 1A) and nerve fiber cells (not shown), Bowman’s glands (Fig. 1B), and the vomeronasal organ (Fig. 1C) in adult mice and mouse pups. Staining for the V-ATPase A subunit in the OE from adult (Fig. 1D) and young (Fig. 1A) animals was predominantly located at the apical region of the cells, including the microvilli of the sustentacular cells. The 70-kDa A subunit expression indicates the widespread presence of the V-ATPase in these structures of the olfactory mucosa, and the strong apical expression of V-ATPase suggests the possibility of transmembrane proton secretion in this tissue by analogy with proton-secreting cell types in other epithelia (34, 35). Adult mice also exhibited V-ATPase A-subunit staining in the lateral membrane domain in a subset of OE cells (Fig. 1D).

V-ATPase expression in the mouse OE was further investigated by immunofluorescence using antibodies against other subunits of this enzyme, including ATP6V1B2 (the B2 isoform of the 56-kDa subunit) and ATP6V1E1 (“E1,” the ubiquitous isoform of the V-ATPase 31-kDa “E” subunit). The expression pattern and subcellular localization of the E1 isoform (Fig. 2A) closely resemble those of the A subunit described above, whether in the mouse pups or adult mice, decalcified or nondecalcified. This is consistent with the presence of fully assembled, functional V-ATPase holoenzymes in the olfactory mucosa. The B2 subunit isoform is considered to be ubiquitous (28, 36) and is expressed predominantly, but not exclusively, on the membrane of intracellular organelles (32). We now report that high levels of B2 expression are detectable in the OE cells (Fig. 2B) and nerve fiber cells, but not in Bowman’s glands (not shown), in adult mice and mouse pups. Figure 2C reveals a difference in the subcellular localization of these two subunits in OE cells. The region of exclusive E1 immunostaining appears to be located closer to the apical pole of the cells.
than the domain in which these subunits are coexpressed. Higher-magnification confocal microscopy images (Fig. 2, D–F) clearly illustrate this point. This is consistent with expression of the E1 subunit alone in the microvilli of the sustentacular cells of the OE, whereas B2 and E1 are both present at the base of these microvilli. This finding suggests that B1, the alternative 56-kDa subunit isoform, may also be expressed in the olfactory mucosa and may be assembled with the other V-ATPase subunits (such as A and E1), instead of B2, in functional H⁺ pumps in the apical membrane domain and apical microvilli.

Expression of the B1 subunit isoform of V-ATPase in mouse olfactory cells. We investigated whether cells in the mouse OE express B1, the B subunit isoform generally associated with transmembrane H⁺ secretion in epithelia (3, 27, 28, 32). We report that OE cells in adults and pups exhibit high expression levels of the B1 isoform, localized mostly to the apical microvilli of sustentacular cells. The V-ATPase B1 subunit was also detected in the lateral membrane domain in a subpopulation of OE cells in adult mice, as reported above for the A subunit (Fig. 1D). B1 was also expressed in Bowman’s glands, but not in nerve fiber cells in the olfactory mucosa (data not shown). Dual-immunofluorescence labeling with the B1 isoform and tubulin emphasized the sharp transition between the pseudostratified columnar OE and the monolayer of respiratory epithelial (RE) cells (Fig. 3A). In the OE, V-ATPase B1-subunit staining appeared much brighter than tubulin staining (which is barely visible in this merged figure). RE cells are characterized by high levels of tubulin expression in their apical cilia. B1-positive RE cells appear similar to previously described lung airway epithelial cells (27) with respect to their tissue distribution and subcellular B1 isoform localization. A transverse cut of the apical membrane domain of the OE (Fig. 3B) revealed that a subset of cells that do not stain for the B1 subunit are interspersed throughout the tissue, appearing as dark circular ~2- to 3-μm-diameter spots. This staining pattern suggests that the sustentacular cells exhibit apical localization of the V-ATPase B1 subunit, whereas olfactory sensory cells, or a subpopulation thereof, do not express this isoform in their apical membrane domain. However, the few cells that express the B1 subunit on their lateral membranes are identifiable as olfactory receptor neurons on the basis of their slender, bipolar appearance (2).

To ensure the specificity of the B1 antibody employed in the present study, we performed a peptide competition assay in adult wild-type mouse OE, as previously described in other organs, such as kidney and epididymis (32). The antibody was preincubated in the presence of a 10-fold excess of peptide against which it had been raised (protein concentration determined by spectrophotometry), and the antibody-peptide mixture was used in parallel to the antibody alone in the same immunofluorescence staining protocol. Images acquired using identical exposure parameters show bright B1 isoform staining in the OE when the antibody was used alone (Fig. 4A), but no specific staining following preincubation with the immunizing peptide (Fig. 4B). This result confirms the specificity of the antibody raised against the B1 subunit of V-ATPase in the olfactory mucosa. The use of B1-deficient [Atp6v1b1−/− (B1−/−)] mice as a negative control further attests to the specificity of this antibody. As expected, no significant B1 immunostaining was detected in the B1−/− mouse olfactory mucosa (see Fig. 8B).

The presence of V-ATPase in general, and the B1 subunit of the enzyme in particular, in the olfactory mucosa was further confirmed by immunoblotting of adult mouse olfactory homogenates. Total kidney homogenate from wild-type [Atp6v1b1+/− (B1+/−)] and B1−/− adult mice was used as control. Olfactory and kidney homogenates blotted with the antibody against the A subunit of V-ATPase exhibit a single, specific band at 70 kDa, as...
expected (Fig. 5). Blotting these samples with the anti-B1 antibody (whole serum) reveals, as previously seen (12, 15), a strong signal at 56 kDa in $B1^{+/−}$ mouse kidney (positive control) that is absent in the $B1^{−/−}$ mouse kidney lane, which is used as negative control. Interestingly, each of the OE lanes shows two bands: a stronger band at the correct size (56 kDa) and a less intense lower-molecular-weight band. Immunoblotting with the same antibody against the B1 isoform preincubated for 1 h at room temperature with the immunizing peptide as described above proved the lower-molecular-weight band to be nonspecific, whereas the 56-kDa band was completely abolished in both OE lanes, as well as in the wild-type mouse kidney lane.

Immunoblotting of the olfactory homogenate samples with olfactory markers was performed to assess their OE content. Results for olfactory marker protein (a 19-kDa protein) and protein gene product 9.5 (a 24.8-kDa protein), well known to

![Fig. 2. Immunocytochemical localization of V-ATPase in mouse olfactory mucosa. A: localization of E1 subunit (red) to apical microvilli of epithelial cells. B: localization of B2 isoform (green) predominantly at the base of the microvilli. C: co-expression of E1 and B2 in subapical domain. D–F: partially distinct regional distribution of these V-ATPase subunits in the apical pole in a different region in the same tissue shown by confocal microscopy at higher magnification. Red E1 subunit staining (D) is clearly more apical than green B2 staining (E), and areas of overlap are also apparent (F). Similar results were found in 9 mice (4 pups and 5 adults, both males and females). Scale bars, 20 μm.](image)

![Fig. 3. Distribution of the B1 subunit of V-ATPase in adult mouse OE. A: localization of B1 (green) to microvilli of sustentacular cells and to lateral membrane in a subpopulation of sensory cells (arrows; also see B) in Cal-Ex-decalcified adult female mouse OE. Extensive apical cilia in respiratory epithelial (RE) cells are stained for tubulin (red), revealing the sharp distinction between OE and RE. A subset of RE cells (arrowheads) show B1 immunostaining that covers most of the region between the apical membrane and the cell nucleus. B: V-ATPase B1-subunit expression pattern in OE being conserved in ImmunoCal-decalcified adult female mouse. Lateral B1 staining is clearly detectable in a subpopulation of sensory cells (arrows). 4,6-Diamidino-2-phenylindole (DAPI) nuclear stain (blue) is also shown. Inset: higher-magnification spinning disk confocal microscopy image showing dark circular spots that correspond to cells lacking apical staining for the B1 subunit. Findings were confirmed in all 15 adult animals (both males and females). Scale bars, 40 μm (A and B) and 5 μm (inset).](image)
be expressed in the olfactory mucosa (13, 21, 22), confirmed their presence in the OE samples used in the present study. As expected, these OE-specific proteins were absent or expressed at a level below our limit of detection in the kidney samples.

Coexpression of the V-ATPase B1 subunit isoform and CA IV. We subsequently attempted to better characterize the subset of olfactory sensory cells that were found to express the B1 subunit of V-ATPase in their lateral membrane domains. We tested the possibility that these cells may be the previously reported CA-rich olfactory neurons (6, 10, 11, 19, 29, 40). CA II expression was investigated to determine whether it is colocalized with the V-ATPase in the mouse OE. Our findings confirm the previously published presence of CA II in a cluster of epithelial cells located in the caudal recesses of the OE (Fig. 6A) and in a very small number of receptor cells outside this region (data not shown). However, no significant levels of lateral B1 immunostaining could be detected in these cells (Fig. 6, B and C). Moreover, even apical staining for the B1 isoform appeared weaker in the region containing the CA II-rich cells. To further assess V-ATPase expression and localization in these cells (Fig. 6D), immunofluorescence staining was performed using antibodies against other subunits of the enzyme, such as A (Fig. 6E) and B2. Similar to the B1 expression, we detected no lateral V-ATPase A-subunit staining in these cells (Fig. 6, E and F), and the apical A-subunit staining appeared less intense than in the neighboring, non-CA II-expressing, regions of the epithelium. The expression pattern of the V-ATPase B2 subunit also replicated these results (data not shown).

We then investigated the expression and localization in the mouse OE of CA IV, a CA isoenzyme that has been described in the OE by immunocytochemistry (40) alongside CA II. Interestingly, we found that most olfactory cells that exhibited lateral staining for the B1 isoform of V-ATPase also expressed detectable levels of CA IV (Fig. 7). To quantify the number of olfactory neurons in which the B1 subunit and CA IV were coexpressed, we counted cells expressing either antigen from three different mice: one nondecalcified, one Cal-Ex-decalcified, and one ImmunoCal-decalcified (on average, n = 30 cells per animal). We report that, overall, 98% of cells that stained laterally for the B1 isoform were also CA IV-positive cells. Conversely, 92% of CA IV-expressing cells exhibited B1 staining localized to their lateral membranes. Thus, we can conclude that the subpopulation of olfactory sensory

![Fig. 4. Peptide competition assay in OE of a nondecalcified mouse using the V-ATPase B1-subunit antibody and the peptide against which it was raised. Anti-B1 antibody (red) was applied alone (A) or after preincubation in the presence of the immunizing peptide (B), in which case the staining was completely abolished. Assay was performed 3 different times in 2 adult males, yielding identical results, and it was further confirmed in 1 female pup. DAPI nuclear stain (blue) is also shown. Scale bars, 20 μm.](image)

![Fig. 5. Detection of V-ATPase subunits and olfactory markers in adult mouse olfactory homogenate by immunoblotting. Twenty-five micrograms of B1+/− mouse OE (lane 1) and 50 μg of B1+/− mouse OE (lane 2) were subjected to SDS-PAGE and blotted with antibodies against V-ATPase A and B1 subunits, olfactory marker protein (OMP), and protein gene product 9.5 (PGP 9.5). Total kidney homogenate from B1+/− (lane 3) and B1−/− (lane 4) mouse was used as control. V-ATPase A subunit was detected in all samples, and B1 was detected in olfactory mucosa and wild-type kidney samples. Immunobiotting of OE samples with antibody against the B1 isoform as whole serum yielded 2 bands: one at 56 kDa and another (less intense) lower-molecular-weight band. Preincubcation of the antibody with the immunizing peptide completely abolished the specific 56-kDa band in all lanes in which it had been detected. Both olfactory markers were expressed in OE, but not in kidney samples.](image)
cells that exhibit lateral staining for the V-ATPase B1 subunit also expresses CA IV.

Expression of the V-ATPase B2 subunit isoform in OE of B1<sup>−−</sup>/ mice. We reported previously that, in B1<sup>−−</sup>/ mice, apical membrane staining for B2, the alternative 56-kDa subunit isoform, is markedly increased in proton-secreting cells of the renal collecting duct (34) and male reproductive tract (12). This is due to relocation of V-ATPase complexes that contain the B2 isoform from the cytosol to the cell plasma membrane to compensate, at least partially, for the absence of B1. This result prompted us to investigate the B2 expression level and pattern in the olfactory mucosa of B1<sup>−−</sup>/ mice.

The immunofluorescence staining of ImmunoCal-decalcified B1<sup>−−</sup> mouse (Fig. 8B) was much brighter compared with the wild-type animal (Fig. 8D). This increase in staining intensity occurred without any detectable change in subcellular localization, as seen in pictures acquired using identical exposure parameters, including exposure time.

DISCUSSION

Our present data show, for the first time, that V-ATPase is expressed in the mouse OE. V-ATPase was detected in olfactory cells, nerve fiber cells, Bowman’s glands, and the vomeronasal organ. The V-ATPase B1 subunit isoform, a marker of specialized proton-secreting cells (3, 28, 32, 36), was found to localize to the microvilli of the apical plasma membrane of sustentacular cells and to the lateral membrane in a subpopulation of olfactory sensory cells. On the other hand, V-ATPase holoenzymes containing the B2 subunit isoform were found to generally assume a more subapical localization in olfactory sustentacular cells. This is reminiscent of other proton-secreting epithelia, such as renal collecting duct A-type intercalated cells (ICs), in which the B1 isoform localizes to the apical membrane and subapical domain, whereas the B2 isoform localization is less polarized, covering the region between the apical membrane and the nucleus and, more generally, the cytosolic domain (32, 34). This is consistent with involvement of B1-containing V-ATPases in regulated membrane H<sup>+</sup> transport and association of B2-containing enzymes with intracellular organelles. By analogy, the two subpopulations of cells that exhibit lateral staining for the V-ATPase B1 subunit also expresses CA IV.
V-ATPases can be expected to fulfill similar functions in the OE. B2 is, however, detectable on the IC plasma membrane and, under certain conditions, can mediate transmembrane H⁺ secretion, such as in B1−/− mice (15, 34). OE cells show an increase in B2 immunostaining intensity in these mice but, in contrast to renal ICs, we detected no localization shift toward the apical pole of the sustentacular cells, aimed at compensating for the lack of the B1 isoform.

On the basis of the localization patterns of the B1 isoform, a dual role can be envisaged for V-ATPase in the OE, depending on the cell type in which the enzyme occurs. Olfactory sustentacular cells have been previously shown to be involved in transmembrane ion transport, since they were reported to express the cystic fibrosis transmembrane conductance regulator, tetracylammonium Cl⁻-sensitive and -resistant K⁺ channels, and two distinct types of Na⁺ (amiloride- and tetrodotoxin-sensitive) channels (17, 26, 37, 42). Thus, the sustentacular cells may be involved in maintaining salt and, possibly, water balance (1, 17) in the neuroepithelial mucous layer. We now suggest that, in addition, these cells can also function via V-ATPase as acid-base regulators of the extracellular environment in the olfactory mucosa. Also, it is conceivable that the pH of the neuroepithelial mucous layer is important for the sensitivity of odor detection, as shown for the concentration of other ions, such as K⁺, Na⁺, and Ca²⁺ (16). Consequently, the V-ATPases expressed in sustentacular cells may play a role in acidifying the mucous layer, which could be important for sensitivity to odorants.

Conversely, when expressed in olfactory neurons, V-ATPase may serve functions related to CO₂ detection and downstream signal transduction. CA II has long been known to be present in rodent olfactory sensory cells (6, 10, 19). CA II production of protons and bicarbonate ions was hypothesized to mediate the opening of cGMP-sensitive cyclic nucleotide-gated channels in a subpopulation of olfactory sensory neurons (19). Interestingly, olfactory neurons with CA activity were recently shown to be CO₂ chemoreceptors (14, 19), and, moreover, CO₂ stimulation was shown to induce variations in mucosal pH (39). Classical freeze-fracture studies revealed the presence of so-called “dumbbell-shaped” or “rod-shaped” intramembranous particles in the apical domain of some OE cells (23, 25), reminiscent of similar particles that are found in proton-secreting cells in various tissues, including the renal collecting duct, epididymis, and amphibian urinary bladder and skin. These specialized intramembranous particles may represent the transmembrane domains of some V-ATPase subunits (8, 31, 43), consistent with our present findings.

Another intriguing finding of the present study is the coexpression of V-ATPase and CA IV in some OE cells. CA IV has been previously located in Bowman’s glands (30) and OE cells, but not in columnar ciliated cells (40). In the kidney, CA IV is found in proximal convoluted tubule cells, where plasma membrane-associated V-ATPase complexes contain the B2 isoform (9), and in A-type ICs of the collecting duct of some species, where B1 is the predominant 56-kDa subunit isoform expressed in plasma membrane-associated V-ATPases (24, 38). By analogy to renal proton-secreting cells, V-ATPases located in olfactory sensory cells can be assumed to mediate proton translocation, this time, in response to CO₂, and, thus, to be involved in modulating the pH of the neuroepithelial mucous layer. Alternatively, by analogy to CA II-expressing olfactory sensory neurons, V-ATPases located in CA IV-expressing olfactory cells are poised to play a role in signal transduction in response to CO₂ stimulation. Functional and behavioral studies are further required to assess the physiological significance of V-ATPase expression and interaction with CA isoforms in the various cell types of the mouse OE.
GRANTS

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