Altered pH\textsubscript{i} regulation and Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{−} transporter activity in choroid plexus of cilia-defective Tg737\textsuperscript{orpk} mutant mouse

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Banizs B, Komlosi P, Bevensee MO, Schwiebert EM, Bell PD, Yoder BK. Altered pH\textsubscript{i} regulation and Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{−} transporter activity in choroid plexus of cilia-defective Tg737\textsuperscript{orpk} mutant mouse. Am J Physiol Cell Physiol 292: C1409–C1416, 2007. First published December 20, 2006; doi:10.1152/ajpcell.00408.2006.—The hydrocephalus phenotype is associated with a marked increase in intracellular cAMP levels in choroid plexus epithelium, which is known to have regulatory effects on ion and fluid movement in many secretory epithelia. To evaluate whether the hydrocephalus in Tg737\textsuperscript{orpk} mutants is associated with defects in ion transport, we compared the steady-state pH\textsubscript{i} and Na\textsuperscript{+}-dependent transport activities of isolated choroid plexus epithelium tissue from Tg737\textsuperscript{orpk} mutant and wild-type mice. The data indicate that Tg737\textsuperscript{orpk} mutant choroid plexus epithelium have lower pH\textsubscript{i} and higher Na\textsuperscript{+}-dependent HCO\textsubscript{3}\textsuperscript{−} transport activity compared with wild-type choroid plexus epithelium. In addition, wild-type choroid plexus epithelium could be converted to a mutant phenotype with regard to the activity of Na\textsuperscript{+}-dependent HCO\textsubscript{3}\textsuperscript{−} transport by addition of dibutyryl-cAMP and mutant choroid plexus epithelium toward the wild-type phenotype by inhibiting PKA activity with H-89. Together, these data suggest that cilia have an important role in regulating normal physiology of choroid plexus epithelium and that ciliary dysfunction in Tg737\textsuperscript{orpk} mutants disrupts a signaling pathway leading to elevated intracellular cAMP levels and aberrant regulation of pH\textsubscript{i} and ion transport activity.

cAMP; ion transport

A GROWING BODY OF EVIDENCE indicates that cilia integrity is required for normal cell function (36). Cilia are complex organelles containing >500 peptides involved in their formation and function (22). There are motile and immotile (primary cilia) forms of cilia that have diverse functions ranging from fluid and cell movement to sensory perception. Motile cilia are found on epithelial cells in the lung and ependyma lining the brain ventricles, while primary cilia are present on most epithelial and nonepithelial cells of the body.

Cilia are assembled through a process called intraflagellar transport (IFT), which mediates the bidirectional movement of large protein complexes (IFT particles) along the microtubule-based axoneme. Anterograde movement from the base toward the tip of the cilium occurs by a kinesin II complex and retrograde from the tip back to the base by a dynein driven complex (35). Polaris is one of the IFT particle proteins and is the product of the Tg737 gene. The Tg737 gene is highly conserved with orthologs in Caenorhabditis elegans (OSM-5), Drosophila (NOMPB), and Chlamydomonas (IFT88) (13, 17, 26, 31, 39). Null mutations in Tg737 or its orthologs in other organisms result in the loss of cilia or flagella (26, 48). In mice, Tg737 null mutations result in midgestation lethality with severe developmental patterning defects (48). In contrast, hypomorphic Tg737 mutations (Tg737\textsuperscript{orpk} mice) that result in stunted and morphologically abnormal cilia cause skeletal patterning defects, polycystic kidney disease (PKD), hydrocephalus, pancreas and liver anomalies, and severe growth retardation with mutants normally dying within 2 wk of birth (3, 9, 24, 39, 47).

A unifying theme of the soft tissue pathologies in Tg737\textsuperscript{orpk} mutants is altered fluid and ion transport properties. Data suggest that such alterations lead to excess cerebrospinal fluid (CSF) accumulation in the ventricles of the brain and cystic lesions in the kidney and impaired fluid movement into the pancreatic duct and biliary tree in the liver. The mechanism by which dysfunction of cilia results in altered fluid transport remains largely unknown. Analysis of this question has been hindered by the embryonic lethality associated with complete cilia loss. Therefore hypomorphic mutations, such as the Tg737\textsuperscript{orpk} mutant mouse, have become an essential resource to begin investigating the mechanisms connecting ciliary dysfunction to disease pathogenesis.

Previously, we described the hydrocephalus phenotype in the Tg737\textsuperscript{orpk} mice. Hydrocephalus is a progressive pathological condition with a diverse etiology. The most common cause of hydrocephalus in humans and murine models is obstruction of the aqueduct that interconnects the brain ventricles. Less frequent causes are overproduction of CSF by choroid plexus and decreased reabsorption by arachnoid granule. Interestingly, the etiology of hydrocephalus in Tg737\textsuperscript{orpk} mice is not associated with duct stenosis or impaired CSF flow but rather with abnormalities in CSF production. The pathology is then aggravated by the disorganized, slow motility of the cilia found on the ependymal cells lining the ventricles (3).

Homeostasis of the aqueous environment of the mammalian brain is maintained by choroid plexus epithelia (CPE) (38). Although, the composition of cerebrospinal fluid is similar to that of serum, it is not a clear ultraltrate but the result of an
active transport mediated by the CPE (8). The apical Na+-K+-APTase is thought to be an important driving force for ion and fluid movement across CPE, which creates a continuous Na+ influx allowing other transporters to use this electrochemical gradient to transport ions and water. Na+-dependent HCO3− transporters play an important role in Na+ and HCO3− secretion into the CSF and maintenance of pHi of the CPE. Currently three Na+-dependent HCO3− transporters have been described in the CPE: electroneutral NBCn1 and NCBE are located on the basolateral membrane and electrogenic NBCe2 on the apical side (7, 27).

In many transport epithelia, ion and fluid secretion is regulated in part by cAMP (2, 11, 12, 44). Alterations in cAMP-dependent fluid movement has been implicated in the pathogenesis of several diseases such as polycystic kidney disease. The increased intracellular cAMP levels in cystic epithelium are associated with excess fluid secretion into the tubular lumen and increased epithelial cell proliferation. These are together thought to be a mechanism leading to cyst formation and expansion. Importantly, approaches to inhibit the increased cAMP levels with vasopressin V2 receptor (V2R) antagonists and expansion. Importantly, approaches to inhibit the increased cAMP levels with vasopressin V2 receptor (V2R) antagonists have been found to ameliorate cystic kidney disease pathology in several mouse models (42, 43).

Previously, we demonstrated that the defects in the cilia on CPE of Tg737orpk mutant mice are associated with markedly elevated levels of intracellular cAMP (3). However, it was not clear whether elevated cAMP levels had any relevance to the hydrocephalus pathology in the mutant animals. In the present study, we begin to address this issue by using ratiometric fluorescence imaging to examine and compare steady-state pHi and Na+−dependent transport activities in the CPE of Tg737orpk and wild-type mice, and we investigate the consequence of changing cAMP levels on these transport activities. Our data suggest that cAMP-mediated signaling is a pathogenic mechanism leading to excess CSF production and the development of hydrocephalus.

MATERIALS AND METHODS

Reagents. The dye BCECF-AM, DIDS, and nigericin were purchased from Molecular Probes (Carlsbad, CA). Dibutyryl-cAMP and H-89 were purchased from Calbiochem (San Diego, CA). All other reagents were obtained from Sigma (St. Louis, MO).

Mice. Tg737orpk mice were generated as described previously (24). The lines were maintained as heterozygous crosses on an inbred FVB/N genetic background. Animals were treated and maintained in accordance with the Institutional Animal Care and Use Committee regulations at the University of Alabama at Birmingham. Genotyping was performed as described previously (46).

Tissue preparation and measurement of pHi. Day 5 and 6 wild-type and Tg737orpk littersmates were euthanized, choroid plexi were removed from the lateral ventricles and placed into a cooled dissection chamber filled with saline solution. Two tissue pieces, freshly isolated from similar regions of choroid plexi obtained from a mutant and wild-type animal, were transferred to a thermo-regulated microscope chamber. The preparations were immobilized with glass micropipettes in a position where the epithelium of the two tissue pieces were facing each other (Fig. 2). This allowed simultaneous imaging of the two preparations. The tissues were then loaded with BCECF by incubating them in a saline solution containing 10 μmol/l BCECF-AM for 20 min. Residual nonhydrolyzed dye was removed before the experiment by flowing saline solution at 2 ml/min for 5 min. During the experiment, the bathing solution was exchanged at a rate of 2.5 ml/min. pHi was measured using a Nikon S Fluor x40 objective and assessed with dual-excitation wavelength fluorescence system, which included a computer-controlled chopper assembly (530-nm emission during alternating 440 and 495 nm light excitation; Photon Technology International, West Sussex, UK) and a cooled SenSys charge-coupled camera (Photometrics, Tucson, AZ). Every experiment was calibrated using two pH points with the high-potassium/nigericin technique as described (41). For each experiment the 495/440 nm ratios were converted to pH. All experiments were performed at 37°C.

Buffering capacity and HCO3− flux. To evaluate acid-base transporter activities, we determined the intrinsic buffering power in both wild-type and Tg737orpk choroid plexus tissues using the weak acid NH4+ (4, 6). These experiments were performed in the nominal absence of CO2/HCO3− and in the absence of external Na+ to minimize the activity of pHi regulatory mechanisms. pHi was measured in response to stepwise changes in bath NH4Cl concentration from 20, 10, 1 to 0 mM, and the intrinsic buffering power was calculated at the mean pH between steps using the following equation:

$$\beta_i = \frac{\Delta[NH_4^+]/\Delta pH}{\Delta pH}$$

where $\beta_i$ is the intrinsic buffering capacity, $\Delta[NH_4^+]$, is the difference in intracellular ammonium concentration (calculated using pKa of 8.9 for NH3+/4 for NH4+) and $\Delta pH$, is the measured change in pH.

As shown in Fig. 1, the intrinsic buffering capacity displayed a linear decrease with higher pHi values. The intrinsic buffering power values at varying pHi were not significantly different in the wild-type and mutant CPE. We therefore pooled the data from both tissues and the intrinsic buffering power (in mmol/l) as a function of pHi was best fit with the equation:

$$\beta_i = -107.54 \times pH_i + 768.03$$

The initial net acid extrusion ($J_{net}$) was calculated using the following formula:

$$J_{net} = dpH_i/dt \times (\beta_c + \beta_{CO_2})$$

where $dpH_i/dt$ is the initial rate of change in pHi (over 30 s) and $\beta_{CO_2}$ is the buffering capacity conferred by bicarbonate, computed from the theoretical relationship

$$\beta_{CO_2} = 2.3 \times [HCO_3^-]$$

Solutions. Table 1 provides the composition of each solution used in our experiments. The pH of the solutions was adjusted to 7.4 at...
37°C. The osmolarity of all solutions was determined with a freezing point depression osmometer and was adjusted with mannitol.

Statistical analysis. Paired Student’s t-test was used for data analysis, where P ≤ 0.05 was considered significant. The data from the intrinsic buffering capacity experiments were analyzed by two-way repeated-measures ANOVA.

RESULTS

Technique of simultaneous imaging of paired tissue preparations. Analysis of physiological parameters, such as pH, can have a high degree of experimental variability as a result of the assay procedures. This can make it difficult to assess the physiological significance of changes that result from experimental or genetic manipulations. Thus we utilized a simultaneous imaging approach (presented in Fig. 2) to directly compare pH in choroid plexus epithelium from mutant and wild-type mice. As shown in Fig. 3, the steady-state pH was found to be higher in CO₂/HCO₃⁻-buffered solutions than in HEPES-buffered solutions, and under both conditions, pH was significantly lower in choroid plexus epithelium obtained from Tg737orpk mutants than from wild-type animals.

Table 1. Composition of experimental solutions used in the study

<table>
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<tr>
<th>Materials, mmol/l</th>
<th>145 NaCl</th>
<th>30 NH₄Cl</th>
<th>0 Na⁺</th>
<th>Na⁺ and HCO₃⁻</th>
<th>0 Na⁺ and HCO₃⁻</th>
<th>0 Na⁺ and 20 NH₄Cl</th>
<th>Nigericin</th>
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<td>120</td>
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<tr>
<td>Choline-HCO₃</td>
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NMDG, N-methyl-D-glucamine.

![Fig. 2. Freshly isolated in vitro choroid plexus preparations from wild-type and Tg737orpk mutant mice visualized simultaneously in one image field. A: bright-field image showing the wild-type and mutant choroid plexus tissues immobilized by micropipettes. Arrowheads indicate the epithelial cells at the edge of the choroid plexus. B: wide-field fluorescence image of the same tissues loaded with the intracellular pH sensitive dye BCECF. White rectangles represent the regions of interest corresponding to the epithelial cells. Scale bar denotes 40 μm.](http://ajpcell.physiology.org/)

![Fig. 3. Measurement of intracellular pH in choroid plexus epithelia. A: representative traces demonstrating steady-state pH in choroid plexus epithelia (CPE) from simultaneously imaged wild-type and Tg737orpk mutant tissues in the absence or presence of CO₂/HCO₃⁻ in the bath. B: graphs showing pH values in CPE in the absence (circles) or presence (squares) of CO₂/HCO₃⁻ from wild-type and mutant animals. Filled symbols denote pH values from individual paired preparations, and open symbols show the average values ± SE (n = 5; the values in the wild-type and mutant groups were different from each other under both conditions).](http://ajpcell.physiology.org/)
wild types could be due to either stimulation of acid-loading or inhibition of acid-extruding mechanisms. Na\(^{+}\)-H\(^{+}\) antiporter is a potent acid extruder involved in regulating pH, and maintaining cell volume in many tissues (5, 15, 30). We therefore examined Na\(^{+}\)-H\(^{+}\) antiporter activity in both mutant and wild-type CPE tissues (Fig. 4). Tissues in the nominal absence of CO\(_2\)/HCO\(_3\) were first acidified using the NH\(_4\)\(^{+}\)-prepulse technique, and pH recovery was blocked by removing external Na\(^{+}\). Na\(^{+}\) was then readded and the initial rate of pH recovery was used as an index of Na\(^{+}\)-H\(^{+}\) antiporter activity. Neither the wild-type nor the mutant CPE tissues showed appreciable increase in pH\(_i\) upon returning Na\(^{+}\) to acid-loaded tissues. Together these data suggest that altered pH\(_i\) is not due to defects of Na\(^{+}\)-H\(^{+}\) antiporter activity.

Na\(^{+}\)-HCO\(_3\)\(^{-}\) cotransport in choroid plexus epithelia from wild-type and Tg737orpk mice. Na\(^{+}\)-HCO\(_3\) cotransporters also play an important role in pH\(_i\) regulation (32). To date, three types of Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transporters have been described in the choroid plexus epithelia. The two basolateral transporters, NCBE and NBCn1, are suggested to play a role in described in the choroid plexus epithelia. The two basolateral transporters, NCBE and NBCn1, are suggested to play a role in

The effect of DIDS on Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport was also determined. We used choroid plexus tissue dissected from the same animal and pretreated one sample with 500 \(\mu\)mol/l DIDS for 15 min. The Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport activity in DIDS-pretreated tissues was 87.3% \pm 19.2% compared with control (\(n = 6\)).

Effect of db-cAMP and H-89 on Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) cotransport in choroid plexus epithelium. An increased level of cAMP has been reported to activate Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport in the pancreas, intestine, and corneal epithelia (2, 40, 45). Also, our previous data have indicated increased levels of cAMP in Tg737orpk CPE (3). Thus we tested the possibility that an increase in intracellular cAMP could stimulate Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport activity in CPE tissue from wild-type mice similar to that seen in Tg737orpk mutants. For this analysis, pairs of CPE tissues were isolated from wild-type mice. To evaluate cAMP responses, one of the tissues was pretreated with 1 mM dibutyryl-cAMP for 20 min before the experiment and the Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport activity was compared with the control CPE. As shown in Fig. 6, A and B, the Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transporter activity was significantly higher in tissues pretreated with db-cAMP.

These data indicate that cAMP may be a modulator that leads to the altered Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport observed in the mutant CPE. To further assess this possibility, we evaluated Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport activity in mutant CPE under conditions where cAMP signaling was inhibited. This was done using H-89, a potent protein kinase A (PKA) inhibitor. As performed with the wild-type CPE explants, a paired prep technique was utilized, where one mutant sample was pretreated with H-89. In all samples analyzed, the preincubation with H-89 resulted in a significantly lower Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport activity compared with the non-treated mutant tissue (Fig. 6, C and D).

Together, these data support a mechanism by which loss of normal cilia function leads to elevated intracellular cAMP levels that cause defects in the regulation of Na\(^{+}\)-dependent HCO\(_3\)\(^{-}\) transport.

DISCUSSION

In our previous work, we demonstrated that Tg737orpk mice develop hydrocephalus that is initiated in the perinatal period and likely involves ion and fluid transport defects across the choroid plexus. This pathology was associated with a marked increase in intracellular cAMP (3). Another phenotype exhibited by the Tg737orpk mutants is the development of cystic renal lesions in the kidney (24). While it is not known whether cAMP is elevated in the kidney of Tg737orpk mutants, it is noteworthy that the cystic kidneys in human ARPKD and ADPKD patients and in many of the PKD mouse models caused by abnormal cilia function do have increased intracellular cAMP. Furthermore, inhibition of cAMP signaling using vasopressin receptor antagonists in mice with PKD greatly improves renal function and pathology and is currently being evaluated as a means of retarding cyst progression (42, 43). Together, these data suggested that cAMP may be central to the initiation of hydrocephalus and other pathological alterations in the kidney, liver, and pancreas of the Tg737orpk mutant mice.

cAMP is known to be an intracellular regulator of ion and water transport in many secretory/reabsorptive epithelia (e.g., pancreas, intestine, cornea, and kidney), and there are data to indicate a role for cAMP in ion and water transport across the CPE (2, 11, 12, 18, 45). In frog CPE, cAMP has been proposed...
to increase HCO₃⁻ secretion and ion transport into CSF; furthermore, in mice, there is an apically localized inward rectifying chloride channel that is activated by cAMP and cAMP agonists (8, 33, 34). Thus, in this work, we evaluated whether altered cAMP levels observed in the CPE might contribute to changes in pHi and ion transport activities that could explain an increase in CSF production and hydrocephalus in the Tg737orpk mutants.

To better compare acid-base transport mechanisms in mutant and wild-type CPE and their responses to cAMP, we utilized an imaging technique with paired tissue preparations where two tissues loaded with BCECF were assayed simultaneously, minimizing variance between experiments. First, steady-state pHi was measured in both mutant and wild-type tissues either in the absence or presence of CO₂/HCO₃⁻. CPE from Tg737orpk compared with wild-type mice had a lower pHi in both buffer conditions. We hypothesized that this was due to altered activity of either acid loading or extruding transporters. Current data indicate that CPE possess a Na⁺/H⁺ antiporter, Na⁺/H⁺-dependent HCO₃⁻ transporters, and a Cl⁻/HCO₃⁻ ex-

![Graph A](image1)

**Fig. 5.** Dependence of pHi on extracellular Na⁺ in CPE following acid load in the presence of CO₂/HCO₃⁻. A: representative traces of Na⁺-dependent pHi recoveries following an intracellular acidification imposed by prior Na⁺ removal for wild-type and Tg737orpk choroid plexus tissues. The initial rate of pH change was measured over 30 s. B: net Na⁺-dependent acid extrusion flux (J_{net}) after an acid load in wild-type and Tg737orpk choroid plexus tissues. Filled circles denote pHi values from individual paired preparations, and open circles show the average values ± SE (n = 5; the values in the wild-type and mutant groups were different from each other).

![Graph B](image2)

**Fig. 6.** Effect of intracellular cAMP concentration on Na⁺-dependent pHi recovery after intracellular acidification in CPE in the presence of CO₂/HCO₃⁻. A: representative traces of Na⁺-dependent pHi recoveries following an intracellular acidification imposed by prior Na⁺ removal for control wild-type (solid line) and db-cAMP treated wild-type (dashed line) choroid plexus tissues. The initial rate of pH change was measured over 30 s. B: net HCO₃⁻ flux after Na⁺ addition in control and db-cAMP-treated preparations (n = 8; the values in the control and db-cAMP-treated groups were statistically different from each other). C: representative traces of Na⁺-dependent pHi recoveries following an intracellular acidification imposed by prior Na⁺ removal for nontreated Tg737orpk mutant (solid line) and H-89 treated Tg737orpk mutant (dashed line) choroid plexus tissues. D: net HCO₃⁻ flux after acid load in nontreated and H-89 treated Tg737orpk mutant choroid plexus tissues. Filled circles denote pHi values from individual paired preparations, and open circles show the average values ± SE (n = 5; the treated and nontreated groups were statistically different from each other).
changer, whose activity could contribute to the altered pH$_i$
regulation (8). Thus a potential caveat that must be noted in this
study is that we do not know whether there is a different
profile of transporters expressed in mice at the ages used to
evaluate CPE transport properties in the Tg737orpk mice.

To begin evaluating the cause of the difference in pH$_i$, we
first analyzed the activity of Na$^+$/H$^+$ exchanger in mutant and
wild-type tissues. However, based on our data, we could not
detect a Na$^+$-dependent pH$_i$ recovery following CPE acidifi-
cation in the nominal absence of CO$_2$/HCO$_3$. This would be
expected if an active NHE were present on the plasma mem-
brane. Thus it is unlikely that this transport would contribute
remarkably to the observed differences between pH$_i$ in CPE
obtained from wild-type and mutant animals. Also, published
data regarding the presence or activity of NHE in CPE are
controversial. In two studies, a basolateral amiloride-sensitive
NHE was suggested to participate in Na$^+$ uptake into CPE,
assuring basolateral Na$^+$ supply in response to apical Na$^+$ flux
into the CSF (10, 25). However, other groups found no evi-
dence for the expression of a Na$^+$/H$^+$ antiporter in choroid
plexus suggesting that this antiporter may not be present or that
another variant may exist (1, 28).

To further investigate the mechanism behind the pH$_i$
differences, we compared Na$^+$/HCO$_3$ cotransporter activity in CPE
from mutant and wild-type animals. There are three Na$^+$/
HCO$_3$ cotransporters on CPE. Two are localized to the baso-
lateral membrane (NBCn1 and NBCe1) while the other is present
on the apical membrane (NBCe2) (7). They all have
important roles in regulating pH$_i$. In addition to pH$_i$ regulation,
the apically localized NBCe2 is thought to contribute directly
to the CSF production (28). In our studies, we found a marked
increase in pH$_i$ and calculated HCO$_3$ flux was present in both
mutant and wild-type samples following Na$^+$ addition to
acidified CPE tissues. In addition, the Na$^+$-dependent HCO$_3$
flux was significantly higher in the mutant vs. wild-type sam-
plens. On the basis of our findings the activity of Na$^+$/HCO$_3$
cotransporter of CPE showed little DIDS sensitivity at low
intracellular pH$_i$.

We also investigated whether intracellular cAMP, which is
markedly elevated in Tg737orpk mutant choroid plexus, was
able to influence Na$^+$-dependent HCO$_3$ transport. cAMP is
known to stimulate HCO$_3$ flux in the cornea, pancreas, and
colon epithelial tissues. However, little is known about cAMP-
mediated HCO$_3$ transport in the mammalian choroid plexus.
In our studies, we found that the addition of db-cAMP to wild-
type CPE does result in significantly higher Na$^+$-dependent
HCO$_3$ flux in acidified CPE tissues compared with untreated
control samples. These data confirm that cAMP is able to
regulate Na$^+$-dependent HCO$_3$ activity in the CPE and sug-
gest that the elevated level of cAMP observed in the mutant
CPE may stimulate Na$^+$-dependent HCO$_3$ transport. To fur-
ther explore this possibility, we next treated mutant CPE
explants with H-89, which blocks cAMP mediated effects
through inhibition of protein kinase A activity. The results
from these experiments indicate that blocking PKA activity
markedly reduced Na$^+$-dependent HCO$_3$ transport in mutant
tissue. Taken together, these data raise the possibility that
aberrant cAMP/PKA-mediated signaling activity is a driving
force in hydrocephalus of Tg737orpk mutants as recently sug-
gested for cyst development in PKD, a phenotype also present
in Tg737orpk animals.

The mechanism by which the impaired ciliary function on
the CPE in the Tg737orpk mutant results in excess CSF is
currently unknown. However, our data suggest at least two
 possibilities. In the first scenario, the cAMP mediated in-
creased Na$^+$-dependent HCO$_3$ transport could be the driving
force that leads to the excess CSF production. Increased
Na$^+$-dependent HCO$_3$ transport activity in the mutants would
cause a net increase in ion transport and subsequent fluid
movement into the CSF. Indeed, data from frog CPE have
already established a connection between cAMP and increase
HCO$_3$ secretion into CSF that does lead to an increase in CSF
production (33, 34). However, we note that the pH$_i$ is low in
our experimental conditions, well beyond the physiological
range, consequently, the direction of the Na$^+$-dependent
HCO$_3$ transport is inward in acidified CPE cells. In vivo, this
is likely different, since at the estimated reversal potential of
about −50 mV the transporter could be driven in either
direction, depending on ion/Vm conditions (23, 29). The lower
pH$_i$ of the mutant CPE could be due to an increased acid
loading by the apical Na$^+$/HCO$_3$ transporter; however, based
on our data, we cannot identify the localization of the Na$^+$/
HCO$_3$ transporter activated by cAMP. We also note here, that
in our experimental conditions, the monitored region of interest
of CP tissue is an intact epithelium, thus epithelial responses to
the change of bathing solution are probably apical events.
However, since our analysis is conducted on relatively small
tissue samples with cut edges, we cannot exclude accessibility
of the bathing solutions to the basolateral side of the CPE,
which could also contribute to the responses seen in our
analyses.

In the second scenario, we also propose a cAMP-driven
effect on ion and water transport across the mutant CPE. In this
case, the increased ion transport would be mediated by the
apically localized inward-rectifying chloride channel (ClC$_2$
like channel). The ClC$_2$-like channel transports both Cl$^-$ and
HCO$_3$ into the CSF and is known to be stimulated by cAMP
(8, 20, 21). Our previous studies have shown that Cl$^-$ levels in
the CSF are elevated in the mutants (3). Thus cAMP-induced
activity of this channel could explain the changes in CSF chloride
levels and lower pH$_i$ observed in the mutants with the altered
Na$^+$-dependent HCO$_3$ transport being a compensatory mecha-
nisms responding to altered pH$_i$. The connection between cAMP
and the activity of these transporters and channels and whether
the altered activity results in increased CSF production in the
Tg737orpk mutants is currently being evaluated.

As indicated above, increased cAMP levels are a pathogenic
factor leading to the development of cystic kidney disease in
ARPKD and ADPKD patients and animal models (42). In the
renal cystic epithelia, adenyly cyclase activity was elevated
through the vasopressin V2 receptor (V2R). Furthermore,
progression of the cystic pathology can be greatly retarded by the
use of V2R antagonists (43). While V2R is not thought to be
expressed in adult CPE, the mRNA was reported in the CPE of
newborn rodents (19). Thus, it would be interesting to deter-
mine whether V2R expression is maintained in mutant CPE,
and whether hydrocephalus pathology can be ameliorated through administration of V2R antagonists.

In summary, our data indicate that loss of normal function of
the ciliogenic protein polaris in Tg737orpk mutant mice results in
a lower steady-state pH$_i$ and higher Na$^+$-dependent HCO$_3$
transport activity in CPE. These changes are associated with
Na⁺-dependent HCO₃⁻ transport in mouse epithelia

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elevated levels of intracellular cAMP in the mutant tissue. Indeed, addition of a cAMP analogue was able to increase Na⁺-dependent HCO₃⁻ transport in wild-type CPE, while H-89, an inhibitor of cAMP-mediated PKA activity, was able to reduce HCO₃⁻ flux in mutant tissue. We are currently evaluating whether the alteration in cAMP-mediated effects on Na⁺/HCO₃⁻ transport activity is associated with increased CSF production that could lead to development of hydrocephalus in these mice.

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REFERENCES


