Polarized expression and function of P2Y ATP receptors in rat bile duct epithelia

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Dranoff, Jonathan A., Anatoly I. Masyuk, Emma A. Kruglov, Nicholas F. LaRusso, and Michael H. Nathanson. Polarized expression and function of P2Y ATP receptors in rat bile duct epithelia. Am J Physiol Gastrointest Liver Physiol 281: G1059–G1067, 2001.—Extracellular nucleotides may be important regulators of bile ductular secretion, because cholangiocytes express P2Y ATP receptors and nucleotides are found in bile. However, the expression, distribution, and function of specific P2Y receptor subtypes in cholangiocytes are unknown. Thus our aim was to determine the subtypes, distribution, and role in secretion of P2Y receptors expressed by cholangiocytes. The molecular subtypes of P2Y receptors were determined by RT-PCR. Functional studies measuring cytosolic Ca2+ (Ca2+o) signals and bile ductular pH were performed in isolated, microperfused in vitro bile duct units (IBDUs). PCR products corresponding to P2Y1, P2Y2, P2Y4, P2Y6, and P2X4 receptor subtypes were identified. Luminal perfusion of ATP into IBDUs induced increases in Ca2+o that were inhibited by apyrase and suramin. Luminal ATP, ADP, 2-methylthio-adenosine 5'-triphosphate, UTP, and UDP each increased Ca2+o at the basolateral domain, but their activation is attenuated by nucleotide hydrolysis. Activation of ductular P2Y receptors mediates functional activation of cholangiocytes.

Multiple subtypes of P2Y receptors are found in mammals, and they are distinguished both by molecular identity and by pharmacological activation (33). In the rat, four P2Y receptors have been identified, designated P2Y1, P2Y2, P2Y4, and P2Y6. Although P2Y2 and P2Y4 are activated by both ATP and UTP, P2Y1 is activated by ADP (and to a lesser extent ATP) and P2Y6 is activated only by UDP. Distinct combinations of P2Y subtypes are found in different epithelial tissues and may allow tissue-specific regulation of nucleotide responses. Moreover, some tissues differentially express specific P2Y receptor subtypes at apical and/or basolateral plasma membranes.

Several lines of evidence indirectly suggest that stimulation of nucleotide receptors may mediate secretion in the liver. Nucleotide release mediates communication from hepatocytes to cholangiocytes in cocultures of isolated cells (39). Furthermore, ATP and other nucleotides are found in bile at concentrations that can activate P2Y receptors (9). Finally, P2Y receptors are found on apical membranes in biliary epithelial cell lines (36, 38). Together, these data suggest that hepatocytes may signal to cholangiocytes through release of nucleotides into bile followed by activation of P2Y receptors at the luminal (apical) membrane. In addition, cholangiocytes also express basolateral P2Y receptors (29), which may mediate signaling from neural or vascular tissue. Thus the goal of this study was to define the expression and subcellular distribution of P2Y subtypes in bile duct epithelia to determine the relative roles of apical and basolateral receptors in mediating functional activation of cholangiocytes.

METHODS

Animals and materials. Male Sprague-Dawley rats (180–250 g; Harlan Sprague-Dawley, Indianapolis, IN) were used for cholangiocyte isolations. Male Fisher 344 rats (225–250 g; Harlan Sprague-Dawley, Indianapolis, IN) were used for bile duct unit experiments. Male Fisher 344 rats were purchased from Harlan Sprague-Dawley and were used for experiments at 4–6 months of age.

EXTRACELLULAR NUCLEOTIDES such as ATP mediate a number of cell processes through interaction with specific P2Y receptors. The expression and subcellular distribution of specific P2Y receptors have been determined by RT-PCR. Functional studies measuring cytosolic Ca2+ (Ca2+o) signals and bile ductular pH were performed in isolated, microperfused in vitro bile duct units (IBDUs). PCR products corresponding to P2Y1, P2Y2, P2Y4, P2Y6, and P2X4 receptor subtypes were identified. Luminal perfusion of ATP into IBDUs induced increases in Ca2+o that were inhibited by apyrase and suramin. Luminal ATP, ADP, 2-methylthio-adenosine 5'-triphosphate, UTP, and UDP each increased Ca2+o at the basolateral domain, but their activation is attenuated by nucleotide hydrolysis. Activation of ductular P2Y receptors mediates functional activation of cholangiocytes.

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P2Y receptors in rat cholangiocytes

Harlan Sprague-Dawley were used for intrahepatic bile duct isolation. ATP, ADP, 2-methylthioadenosine 5’-triphosphate (2-MeS-ATP), adenosine 5’-O-(3-thiotriphosphate) (ATP-γ-S), α,β-methyleneadenosine 5’-triphosphate (α,β-MeATP), β,γ-methyleneadenosine 5’-triphosphate (β,γ-MeATP), 2’- and 3’- O-(4-benzoylbenzoyladenosine 5’-triphosphate (BzATP), UTP, UDP, ACh, suramin, apyrase, and 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA)-AM were obtained from Sigma Chemical (St. Louis, MO). Fluo 4-AM and 2’,7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) dextran were obtained from Molecular Probes (Eugene, OR). All other chemicals were of the highest quality commercially available.

Buffer composition. The composition of isotonic (290 mosmol/kgH2O) Krebs-Ringer-bicarbonate buffer (KRB) was (in mM) 120.0 NaCl, 5.9 KCl, 1.2 NaHPO4, 1.0 MgSO4, 25.0 NaHCO3, 1.25 CaCl2, and 5.0 glucose. For HCO3⁻ transport experiments, the perfusion buffer contained 140 mM NaCl, 5 mM NaHPO4, and 25–100 μM BCECF dextran, pH 7.2.

Preparation of single rat bile duct cells. Single cholangiocytes were prepared and characterized in the Cell Isolation and Morphology Core Facilities of the Yale Liver Center as described previously (40). This preparation results in a bile duct epithelial preparation that is ~98% pure as assessed by positive staining for the biliary epithelial markers γ-glutamyl transpeptidase, cytokeratin-19, and cytokeratin-7 (1, 34). Experiments using single bile duct cells were performed 12 h after plating.

RT-PCR. P2Y receptor subtypes in cholangiocytes were detected using two-step RT-PCR performed on RNA from freshly isolated bile duct cells. Bile duct cell total RNA was extracted using TRizol reagent (Life Technologies, Rockville, MD). RNA was analyzed by electrophoresis and spectrophotometry and treated with RNase-free DNase (RQ1, Promega, Madison, WI) for 15 min at 37° before RT-PCR. First-strand cDNA was prepared according to manufacturer’s instructions using Moloney murine leukemia virus reverse transcriptase and random hexamer and oligo(dT)₁₈ primers (Advantage RT-for-PCR; Clontech, Palo Alto, CA). First-strand cDNA was used as a template for specific amplification of primers for P2Y subtype pairs: P2Y₁, 5’-TG CG GTT GGT GCT GCA CCC TCT CAA GCT-3’ and 5’-CGG GAC AGT CTC CTT CTG AAT GTA-3’; P2Y₂, 5’-CTG CCA GGA ACC CGT GCT CTA CTT-3’ and 5’-CTG AGG TCA AGT GAT-3’; P2Y₄, 5’-CAG CCA GTA CTG GTC AGT-3’; P2Y₅, 5’-GGA GAG TCT GTC TGC TGC CGT GTA-3’ and 5’-GAG ACA GAG ACA GTA AGC AC-3’; P2Y₆, 5’-GGA GCC CTG TGC TGC CGT GTA-3’ and 5’-TAC CAC GAC AGC CAT ACC GCG CGC-3’(44); P2X₁, 5’-AGG CCG TGT GGG GGT TTC TTC TC-3’ and 5’-ACC TGG CCT TCT TTT TTT TC-3’; P₂X₂, 5’-GAG GCG GGT CAA GGG CGG TCT G-3’ and 5’-GGG GTC TGG GGA TCG TCC TCT AAT TC-3’; P₂X₅, 5’-CCG GCC ACC GAG CAG GCT ATG TGC G-3’ and 5’-AAG GCC ACC GAG CAG GCT ATG TGC G-3’; P₂X₆, 5’-GAT CAT GGA CTT GGT TCC TGC CGC TGC CGT GTA-3’ and 5’-TAC CAC GAC AGC CAT ACC GCG CGC-3’(44); P₂X₇, 5’-CGG TGT GGG GGT TTC TTC TC-3’ and 5’-ACC TGG CCT TCT TTT TTT TC-3’; P₂X₈, 5’-GAG GCG GGT CAA GGG CGG TCT G-3’ and 5’-GGG GTC TGG GGA TCG TCC TCT AAT TC-3’; P₂X₉, 5’-ACC TGG CCT TCC TCT AAT TC-3’ and 5’-AAG GCC ACC GAG CAG GCT ATG TGC G-3’. Experiments using single bile duct cells were performed 12 h after plating.

Measurement of nucleotide-induced changes in cytosolic Ca²⁺ in IBDUs. Changes in cytosolic Ca²⁺ (Ca²⁺) in cholangiocytes were detected using the cell-permeant Ca²⁺-sensitive fluorescent indicator fluo 4-AM. After dissection and perfusion as described in Microperfusion of microdissected rat intrahepatic bile duct units, IBDUs were luminally perfused with fluo 4-AM (24 μM) for 30 min at 37°C. A fluorescence illuminator (90720 TE epifluorescence attachment for Nikon TE 300 microscope) was specially modified by the Mayo Division of Engineering to detect fluorescence changes from a fixed-size aperture. Fluorescence was detected from a 100-μm-diameter circular spot within a region of cytosolic fluorescence in an IBDU by a photosensor module (Hamamatsu Photonics, Bridgewater, NJ). Background fluorescence and autofluorescence were negligible with this system. The signal was digitized by a data-acquisition board (PCI-6023E, National Instruments, Austin, TX) using a P-type computer. A custom-modified software application (LabView 5.1, National Instruments) was used to control data acquisition and data display during each experiment and to output the data to a spreadsheet for analysis. Data samples were collected 10 times/s. Apical P2Y and P2X receptors were stimulated by perfusion of nucleotides into the duct lumen, whereas basolateral P2Y and P2X receptors were stimulated by addition of nucleotides to the perfusing bath fluid. Fluorescence values were expressed as percentage of baseline.

Data analysis of Ca²⁺ changes. Each experiment was plotted separately with a spreadsheet program, with time as the x-axis and raw fluorescence as the y-axis. Baseline and peak values were identified manually. Baseline fluorescence was defined as a plateau of fluorescence lasting ≥5 s immediately before addition of agonist. Experiments were not performed unless a constant baseline of 10–20 s was identified before experimental runs. Isolated fluorescence peaks lasting ≤1 s were disregarded to eliminate the possibility of artificial fluorescence increases. Fluorescence increases for
each individual experiment were expressed as a peak-to-
baseline fluorescence ratio.

Measurement of nucleotide-induced changes in pH in
IBDUs. The luminal pH of perfused IBDUs was measured by
using the cell-impermeant pH-sensitive dye BCECF dextran
and the quantitative epifluorescence approach described
above (27). The IBDUs were perfused with HCO3−-free buffer
containing BCECF dextran (pH 7.2) and simultaneously
bathed with KRB or HCO3−/CO2-free buffer (pH 7.4). Luminal
dye was excited alternately at 495 ± 10 nm and 440 ± 10 nm
wavelengths, and emission was monitored as described
above. In situ calibration curves were generated to convert
fluorescence excitation ratios (F495/F440) to pH values.

Statistics. Changes in fluo 4 fluorescence ratio and pH are
expressed as means ± SE. Comparisons between groups
were made using Student’s t-test. A P value of <.05 was
taken as significant.

RESULTS

Rat cholangiocytes express multiple P2 receptor sub-
types. Molecular expression of P2Y receptor subtypes in
freshly isolated rat cholangiocytes was determined by
RT-PCR. Oligonucleotide primers specific to the four
P2Y subtypes cloned in rat (33, 44) were used. Cholan-
giocytes expressed all four of these P2Y subtypes (Fig.
1), which were absent in RNA and water controls.
Similar results were found in freshly isolated rat hepa-
tocytes (not shown). These results are in contrast to
findings in the normal rat cholangiocyte (NRC) cell
line, in which the only P2Y receptor molecularly iden-
tified was the P2Y2 receptor (38). Because nucleotide-
induced increases in Ca2+ could be induced by P2X
ligand-gated ion channels as well as P2Y G protein-
coupled receptors, we tested for the presence of P2X
receptors in rat cholangiocytes. Molecular expression of
P2X receptors was examined using RT-PCR of
immunoisolated rat cholangiocytes with oligonucleotide
probes specific for rat P2X subtypes P2X1–7 (see Fig.
6A). Cholangiocytes expressed only the P2X4 subtype.
Positive control PCR products were expressed using
either total liver (P2X1–7) or heart (P2X1,2,4,5) (not
shown). Together, these data show that rat cholangio-
cytes express P2Y1, P2Y2, P2Y4, and P2Y6 receptors
and P2X4 receptors.

Rat cholangiocytes express multiple P2Y subtypes at
apical plasma membrane. Experiments were performed
in IBDUs to determine which of these P2Y receptors
were expressed at the apical membranes. IBDUs were mounted on a specially designed stage for
visualization on a fluorescence microscope (Fig. 2A).
Because P2Y receptors couple to phospholipase C
(PLC)- and inositol trisphosphate (InsP3)-mediated in-
creases in Ca2+, Ca2+ was monitored to indicate P2Y
receptor activation. IBDUs were loaded with the Ca2+-
sensitive dye fluo 4-AM by luminal perfusion with the
dye (Fig. 2B). Subsequent luminal perfusion with nu-
cleotides induced serial increases in fluo 4 fluorescence,
reflecting increases in Ca2+ (Fig. 2C). These findings
suggest that P2Y receptors are expressed on the apical
membrane of bile duct cells.

To verify that Ca2+ increases induced by luminal
nucleotides were specific to P2Y receptor activation,
experiments were performed in the presence of either
the ATP diphosphohydrolase apyrase or the P2Y inhib-
itor suramin (Fig. 3). ATP (100 μM) increased fluo 4
fluorescence by 78 ± 10% (n = 5), whereas ATP (100
μM) + apyrase (3 U/ml) increased fluorescence by only
5 ± 0.8% (n = 5; P < 0.01 by 1-tailed t-test), and ATP
(100 μM) + suramin (50 μM) increased fluorescence by
only 9 ± 2.7% (n = 5; P < 0.02 by 1-tailed t-test). These
findings demonstrate that increases in Ca2+ induced
by luminal perfusion of nucleotides are caused by ac-
activation of apical P2Y receptors.

To determine whether apical cholangiocyte P2Y rece-
ptors are activated in a concentration-sensitive fash-
ion, we perfused IBDUs with ATP at a concentration
range of 10 nM–1 mM. Perfusion with 10 nM ATP did
not increase fluo 4 fluorescence; 100 nM ATP increased
fluo 4 fluorescence by ~22%; 1 μM and 10 μM ATP
increased fluo 4 fluorescence by 41% and 46%, respec-
tively, and 100 μM ATP increased fluo 4 fluorescence by ~88% and was the maximum concentration of ATP
found to increase fluo 4 fluorescence. Perfusion with 1
mM ATP decreased fluo 4 fluorescence by ~5%. This
may have been caused by hyperstimulation of G pro-
tein-coupled receptors leading to nonspecific G protein
cross talk (32) or, alternatively, by acidification of the
cytosol and subsequent pH-dependent decrease in fluo
4 fluorescence (Molecular Probes). Together, these
findings suggest that cholangiocytes are sensitive to

Fig. 1. A: rat cholangiocytes express P2Y receptor subtypes P2Y1,
P2Y2, P2Y4, and P2Y6. Molecular expression of P2Y subtypes by rat
cholangiocytes was evaluated by RT-PCR. Cholangiocytes were
immunoisolated, and RNA was extracted. Cholangiocyte cDNA was
exposed to specific oligonucleotide primers for P2Y1 (lane 1), P2Y2
(lane 2), P2Y4 (lane 3), and P2Y6 (lane 4). Bands of predicted molec-
ular weight are found for each subtype but not in RNA controls not
exposed to reverse transcriptase (−, lanes 5–8). Positive bands were
gel excised and sequenced and were found to have 100% identity to
published sequences. B: rat cholangiocytes express the P2X receptor
subtype P2X4. Molecular expression of subtypes P2X1–7 was exam-
ined using RT-PCR of immunoisolated rat cholangiocytes using spe-
cific oligonucleotide probes (P2X2 oligonucleotides were used as
a positive control in lane 2). Cholangiocytes expressed subtype P2X4.
Positive bands were found in total liver (P2X1–7) and heart
(P2X1,2,4,5).
ATP over the range 100 nM–100 μM, which includes the concentrations of nucleotides found in bile (9).

To investigate whether apical cholangiocyte membranes express multiple subtypes of P2Y receptors, we perfused IBDUs with nucleotides that are specific to each particular P2Y receptor subtype. ATP, ADP, 2-MeS-ATP, UTP, and UDP each increased fluo 4 fluorescence by 41% to 62% at concentrations of 1–10 μM and by 88% to 100% at a concentration of 100 μM (Fig. 4B). Pretreatment of ducts with ATP, ADP, UTP, or UDP had no effect on subsequent nucleotide responses (ATP, n = 6; ADP, n = 6; UTP, n = 7; UDP, n = 7). Because ADP, ATP, and 2-MeS-ATP act at the P2Y1 receptor, UTP and ATP act at the P2Y2 and P2Y4 receptors, and UDP acts at the P2Y6 receptor, these findings suggest that rat cholangiocytes express all four of these P2Y subtypes at the apical membrane. Because nucleotide pretreatment had no effect on subsequent nucleotide responses, this suggests that these responses to different nucleotides are mediated by expression of multiple P2Y receptor subtypes rather than nucleotide interconversion.

Rat cholangiocytes express P2Y receptors at basolateral plasma membrane. Additional experiments were performed in IBDUs to investigate whether P2Y receptors are expressed at the basolateral membranes of cholangiocytes. The presence of basolateral P2Y receptors was investigated by addition of nucleotides to the perifusing bath. ACh was used as a positive control because cholangiocytes are known to express M3 muscarinic receptors at the basolateral membrane (2, 29). ACh (100 μM) increased fluo 4 fluorescence by 24% (n = 4; P < 0.05 vs. ATP), but only minimal increases in fluorescence (2–10%) were evoked by ATP (100 μM; n = 5), ADP (100 μM; n = 5), UTP (100 μM; n = 5), or UDP (100 μM; n = 5). However, basolateral addition of the nonhydrolyzable ATP analog ATP-γ-S (100 μM) increased fluo 4 fluorescence by 30% (n = 3; P <...
ACh, similar to the increase induced by ACh (Fig. 5). The lowest concentration of ACh that induced an increase in fluo 4 fluorescence was 10 μM, consistent with previous observations in cholangiocytes (29), whereas the lowest concentration of ATP-γ-S that induced an increase in fluo 4 fluorescence was 1 μM. The effect of ATP-γ-S was inhibited by both the P2Y antagonist suramin (50 μM) and the Ca^{2+} chelator BAPTA-AM (50 μM).

Together, these data suggest that cholangiocytes express P2Y receptors at the basolateral membrane, but activation of these receptors by ATP and other nucleotides is attenuated in part by hydrolysis of nucleotides. Moreover, the decreased effect of basolateral ATP-γ-S (relative to apical nucleotides) suggests that the potency of nucleotides at the basolateral membrane is weaker than at the apical membrane. Therefore, expression of P2Y receptors at the basolateral plasma membrane may be lower than that at the apical membrane. Alternatively, apical P2Y receptors may couple more strongly to G proteins that link to PLC and InsP₃ formation (31). A final possibility is that the unique ability of basolateral ATP-γ-S to induce a relatively weak Ca^{2+} transient is caused by phosphatase-resistant thiophosphorylation reactions catalyzed by ectokinases rather than activation of basolateral nucleotide receptors. Direct measurement of basolateral ectonucleotidase activity would be needed to distinguish among these possible explanations.

**Functional expression of P2X receptors in rat cholangiocytes.** Functional expression of P2X receptors was examined using P2X-specific synthetic nucleotides α,β-MeATP (P2X agonist most potent at P2X₁ and P2X₂), β,γ-MeATP (P2X agonist most potent at P2X₁), and BzATP (P2X agonist most potent at P2X₁ and P2X₇) (Fig. 6) (30). Stimulation of IBDUs with these agonists at either the apical or basolateral membrane induced minimal changes (~6% to +8%) in Ca^{2+}, yet apical perfusion with ATP as a positive control yielded Ca^{2+} increase of 46 ± 6.8%, similar to what was seen in prior experiments. These data suggest that, although rat cholangiocytes express the P2X receptor subtype P2X₄, they do not express P2X subtypes sensitive to P2X-
specific nucleotides. Because there are no specific agonists available for the P2X4 receptor, the polarity of its expression cannot be established.

Activation of bile duct epithelial P2Y receptors induces bile duct alkalization. To determine the functional role of cholangiocyte P2Y receptors in fluid and electrolyte secretion, changes in biliary pH were monitored after addition of ATP-g-S to either the apical or the basolateral cell surface (Fig. 7). ATP-g-S was used to eliminate the role of nucleotide hydrolysis seen in Fig. 5. Apical perfusion of ATP-g-S (100 μM) induced a net increase in biliary pH of 0.32 ± 0.078 units (P = 0.039). Basolateral perfusion of ATP-g-S (100 μM) induced a trend toward increase in biliary pH of 0.10 ± 0.019 units (P = 0.058). The finding that apical ATP-g-S induced a larger increase in pH than basolateral ATP-g-S is consistent with the difference in Ca2+ increases seen with these agonists. For comparison, stimulation with a maximal concentration of forskolin increases luminal pH by ~0.6 units in this experimental system (27). These experiments demonstrate that nucleotide signaling may contribute to regulation of HCO3 secretion in bile duct epithelia.

DISCUSSION

The role of extracellular nucleotides as signaling molecules has now been shown in many tissue types (15, 28, 42, 46). A specific role for nucleotides and their
receptors in liver, and in cholangiocytes in particular, has been demonstrated in several ways. First, hepatocytes can signal to cholangiocytes over distances of several hundred micrometers through nucleotide release, suggesting that P2Y receptors allow paracrine signaling between these two liver cell types (39). Second, nucleotides are found in nanomolar to micromolar concentrations in bile, suggesting that purinergic signaling from hepatocytes to cholangiocytes may occur through release of nucleotides into bile (9). Third, expression of nucleotide receptors by bile duct-derived cell lines is critical for autoregulation of cell volume, demonstrating that P2Y receptors are important in autocrine signaling in these cells (35). Finally, bile duct-derived cell culture models express apical P2Y receptors, which would be necessary for activation by nucleotides released into bile (36, 38). Together, these observations show an important potential role for purinergic signaling through P2Y receptors in bile ductular signaling.

However, previous studies failed to address several key questions. First, the distribution and relative roles of the different subtypes of P2Y receptors found in cholangiocytes were unknown. The various subtypes of P2Y receptors expressed in rat and other mammals differ not only in molecular structure but also in nucleotide pharmacological preference. Here we found that rat cholangiocytes express all four of the P2Y subtypes previously identified in this species. Thus these cells can be activated not only by ATP but also by a variety of nucleotides. This finding is important for several reasons. First, multiple P2Y subtypes have been cloned only in the past few years. Although these receptors share common tertiary structure and function, their primary structures vary greatly. A precise understanding of P2Y receptor tissue distribution would facilitate design of pharmacological agonists or antagonists for these receptors. Moreover, endogenously occurring nucleotides found in body fluids include not only adenosine nucleotides but uridine nucleotides as well (21). Because all nucleotides are equipotent in cholangiocyte activation, it is possible that those nucleotides are also involved in signaling responses for these cells.

A second question that has been addressed in the present study is the distribution of P2Y receptors in native cholangiocytes in particular. Although the demonstration of P2Y receptors has been examined in cholangiocyte cell lines (36, 38), cell lines in general have been inaccurate as models of P2Y receptor expression. Specifically, it has been found that expression and polarized distribution of P2Y receptor subtypes depends on both number of days in culture and cell support mechanisms (18). In fact, the NRC bile duct cell line appears to express only P2Y2 receptors at the apical membrane (38), whereas we report here that native cholangiocytes also express P2Y1, P2Y4, and P2Y6 apically. Thus cell culture models may provide an insufficient means to determine the polarized distribution of P2Y receptor subtypes in native tissues. In the liver, this is of critical import—if receptors are expressed apically, they are activated by nucleotides released directly into bile, whereas if they are expressed basolaterally, they are activated by nucleotides released into blood or by nerves. Our finding that P2Y receptors are expressed both apically and basolaterally shows that each mechanism may be operative in bile duct epithelia. Our finding that basolateral nucleotide signaling is attenuated at least in part by nucleotide hydrolysis is consistent with the observation that ATP degradation is biphasic during basolateral exposure to NRC cells (36). This also suggests that nucleoside triphosphate diphosphohydrolases may be localized to the basolateral membranes of cholangiocytes rather than to nearby cells or interstitium. Because nucleotides appear to be degraded more quickly basolaterally, P2Y receptor activation at this plasma membrane domain may be more important for autocrine signaling, whereas activation of apical P2Y receptors may be more important for paracrine signaling (Fig. 8).

Because P2Y receptor activation can induce Cl− and HCO3− secretion independent of CFTR, these receptors are an attractive therapeutic target in secretory disorders such as CF. In fact, nucleotides activate Cl− currents in multiple tissues affected by CF, including respiratory, reproductive, and digestive epithelia (7, 12, 24, 26, 41, 48). In cholangiocytes, both CFTR and P2Y-activated non-CFTR Cl− channels are thought to induce bulk fluid and electrolyte secretion through apical Cl−/HCO3− exchange, duct alkalization, and paracellular water movement. Furthermore, second messengers such as cAMP and Ca2+ may induce ATP release (5, 6, 9) and thereby activate P2Y-mediated secretion. Activation of Ca2+-sensitive Cl− channels and subsequent Cl−/HCO3− exchange is a likely mechanism for nucleotide-induced bile duct alkalization. However, duct alkalization may also work through direct HCO3− export (11, 14). Furthermore, studies in the intact, bivascularly perfused liver suggest that regulation of biliary HCO3− excretion may be the principal function of cholangiocytes in vivo (17). The current work provides evidence that apical stimulation with nucleotides may thus have the same physiological effect as basolateral stimulation with secretin or ACh (17). The mechanism by which extracellular nucleotides regulate bile duct alkalization should provide a robust area for further research that may provide insights into the pathogenesis of CF and other cholestatic conditions in which cholangiocytes are the target of disease.

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