0026-895X/03/6304-773–776$7.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 63:773–776, 2003

Vol. 63, No. 4
2376/1051944
Printed in U.S.A.

PERSPECTIVE

Knock-Out Mice Reveal Tissue-Specific Roles of P2Y Receptor Subtypes in Different Epithelia

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Received December 23, 2002; accepted January 3, 2002

This article is available online at http://molpharm.aspetjournals.org

ATP, UTP, and their corresponding disphosphates function as intercellular signaling molecules when released to, or generated within, extracellular compartments. Genes encoding eight G protein-coupled P2Y nucleotide receptor subtypes (von Kugelgen and Wetter, 2000), seven ionotropic P2X nucleotide receptor subtypes (North, 2002), and at least nine different ecto-nucleotidases (Zimmermann, 2000) have been identified in human and other vertebrate genomes. Most mammalian cell types express one or more subtypes of nucleotide receptor together with various combinations of the ecto-nucleotidases used for degrading and/or interconverting extracellular nucleotides. In this issue of *Molecular Pharmacology*, Robaye et al. (2003) describe the generation and initial phenotypic characterization of P2Y4 receptor null mice. Their findings demonstrate that the P2Y4 receptor is the dominant UTP- and ATP-sensitive regulator of salt and fluid transport in the jejunum of the small intestine. Full appreciation of the significance of these findings might be aided by a brief overview of nucleotide-based signaling in epithelial tissues.

Most of the eight mammalian P2Y receptor subtypes are expressed in a broad range of tissues and cell types. However, epithelia and cells derived from the airways, gut, kidney, and exocrine glands have proven particularly significant as examples of tissues that express multiple subtypes of seemingly redundant—with regard to G protein coupling and second messenger generation—P2Y subtypes that are differentially used for the regulation of distinct tissue-specific functions. Table 1 summarizes the pharmacological properties of the eight mammalian P2Y receptor subtypes as recently reviewed in *TIPS* (Abbracchio et al., 2003). Based on their functional coupling to particular G proteins and effector proteins, P2Y receptors can be broadly subdivided into the five G_q-coupled subtypes (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11) and three G_i-coupled subtypes (P2Y12, P213, P2Y14). The latter grouping includes the recently cloned UDP-glucose receptor (Chambers et al., 2000) as P2Y14 on the basis of its sequence homology to P2Y12 and P2Y13 (Abbracchio et al., 2003). It should also be noted that P2Y11 receptors can additionally couple to G_s and activate adenylyl cyclase (Zambon et al., 2001). Expression of the five G_q-coupled P2Y receptor subtypes has been documented at the molecular, functional, and pharmacological levels in a variety of epithelial tissues and epithelial cell types (von Kugelgen and Wetter, 2000). Although mRNA for P2Y12 (Hollopeter et al., 2001), P2Y13 (Communi et al., 2001), and P2Y14 (Chambers et al., 2000) receptors is present in a variety of epithelial tissues, bona fide functional expression of these P2Y subtypes in epithelial cell types has not yet been described.

Figure 1 illustrates the potential or, in some cases, documented complexity of nucleotide-based signal transduction and regulation in epithelial tissues. Certain P2Y receptor subtypes, such as P2Y4 and P2Y6, are predominantly trafficked to apical plasma membrane domains (Homolya et al., 2000; Sage and Marcus, 2002), whereas P2Y1 (Homolya et al., 2000) and P2Y11 receptors (Nguyen et al., 2001; Zambon et al., 2001) seem generally localized in the basolateral plasma membranes of those epithelia in which these receptors are expressed. In contrast, P2Y2 receptors have been characterized in apical and/or basolateral membranes of diverse epithelia (Homolya et al., 2000; Sage and Marcus, 2002). Most epithelial cell types express at least one apical P2Y subtype as well as a pharmacologically distinct basolateral subtype: 1) apical P2Y4 versus basolateral P2Y2 in vestibular dark cells of the inner ear (Sage and Marcus, 2002); 2) apical P2Y2 versus basolateral P2Y11 in pancreatic duct epithelial cells (Nguyen et al., 2001); 3) apical P2Y2 and P2Y6 versus basolateral P2Y1 and P2Y2 in certain airway epithelial cells (Homolya et al., 2000). Moreover, different combinations of P2Y receptors may be expressed within the multiple cell types (e.g., mucous-secreting cells and serous cells in airways and absorptive cells, goblet cells, and enteric

ABBREVIATIONS: SCC, short-circuit current; PI-PLC, phosphatidylinositol-specific phospholipase C; CFTR, cystic fibrosis transmembrane regulator.

TABLE 1
Pharmacological properties of P2Y receptors

Receptor Subtype	G protein-Effector Cascade	Selectivity for Physiological Nucleotide Agonists	Functional Expression in Epithelial Tissues
P2Y1	Gq → PI-PLC	ADP > ATP ≫ UDP, UTP	Airway, exocrine
P2Y2	Gq → PI-PLC	ATP = UTP ≫ ADP, UDP	Airway, exocrine, kidney, inner ear
P2Y4	Gq → PI-PLC	UTP ≫ ATP, UDP, ADP (human) UTP = ATP ≫ UDP, ADP (rodent)	Small intestine, inner ear
P2Y6	Gq → PI-PLC	UDP > UTP > ADP ≫ ATP	Gallbladder, airway
P2Y11	Gq → PI-PLC and Gs → AC	ADP > ATP ≫ UDP, UTP	Exocrine, kidney
P2Y12	Gi → AC/others	ADP > ATP ≫ UDP, UTP	?
P2Y13	Gi → AC/others	ADP > ATP ≫ UDP, UTP	?
P2Y14	Gi → AC/others	UDP-glucose ≫ UTP, ATP, UDP, ADP	?

endocrine cells in intestinal microvilli) that comprise complex epithelial tissues. Defining how extracellular nucleotides and different P2 receptors modulate epithelial function is further complicated by the ability of ecto-nucleotidases to serially convert the nucleotide agonist for one P2Y receptor subtype into agonists for other P2Y and/or adenosine receptors (Huang et al., 2001). In some epithelia, direct stimulation of the G_q-coupled, Ca^{2+}-mobilizing P2Y receptor subtypes can also indirectly elicit cAMP-regulated cellular functions via activation of a phospholipase A_2 → cyclooxygenase → prostaglandin E release → EP receptor autocrine/paracrine loop (Post et al., 1996). Although not illustrated in Fig. 1, some epithelia additionally express P2X family ATP-gated ion channel receptors that can also contribute to the modulation of epithelial ionic fluxes by extracellular nucleotides (North, 2002).

This rather convoluted web of nucleotide-based signal transduction in epithelia provides the general context for the analysis of P2Y4 receptor function described by Robaye et al. (2002). Elucidation of specific physiological roles for particular P2Y and P2X receptor subtypes in epithelial (and other) tissues has been significantly hindered by the general absence of high-affinity antagonists that exhibit suitable subtype selectivity. Most of the information and insights summarized in the Fig. 1 overview have been gleaned via a combination of indirect pharmacological approaches coupled with molecular analysis of P2Y subtype expression patterns. Given this dearth of subtype-selective antagonists, it is not surprising that transgenic approaches have been employed to generate knockout mice that lack expression of particular nucleotide receptors. This has been an especially fruitful approach in the case of several ionotropic P2X receptors, including P2X1 (Mulryan et al., 2000), P2X3 (Zhong et al., 2001), and P2X7 (Labasi et al., 2002). With the exception of studies demonstrating marked alterations in hemostatic and thrombotic responses in mice bearing targeted deletions in the genes encoding the P2Y1 receptor (Fabre et al., 1999) and P2Y12 receptor (Foster et al., 2001), there is less information regarding the in vivo phenotypic consequences of deleting particular P2Y subtypes. However, Cressman et al. (1999) have described elegant in vitro measurements of short-circuit current (SCC) in several epithelial tissues (trachea, gallbladder, and small intestine) freshly isolated from P2Y2-null mice. Those experiments clearly established the latter receptor as the major UTP- and ATP-sensitive regulator of salt and fluid transport in airway epithelial cells given the >90% reduction (compared with wild-type P2Y2 +/+ tissue) in SCC response to exogenous ATP or UTP that characterized the P2Y2-null epithelia. In contrast, analysis of jejunal segments from P2Y2 −/− small intestines revealed wild-type SCC responses to both ATP and UTP. Both the wild-type and P2Y2 −/− jejuna were similarly unresponsive to UDP. Thus, the studies of Cressman et al. (1999) indicated that jejunal epithelial cells expressed a P2Y subtype other than P2Y2 that, nonetheless, exhibits a P2Y2-like selectivity order for nucleotide agonists (i.e., ATP = UTP >ADP, UDP). By demonstrating that ATP- and UTP-induced SCC responses are abolished in jejuna derived from P2Y4-null mice, Robaye et al. (2003) have confirmed the most straightforward interpretation of the Cressman et al. findings: that the P2Y4 receptor, rather than P2Y2 receptor, is the dominant UTP-and ATP-sensitive regulator of salt and fluid transport in the jejunum of the small intestine.

Why two P2Y receptor subtypes with very similar agonist selectivities are differentially expressed in the apical membranes of different epithelial tissues from the same organism remains an intriguing question. The question is even more perplexing when one considers that these particular P2Y subtypes are redundant (superficially, at least) with respect to major pathways of G protein coupling and second messenger generation. When heterologously expressed in 1321N1 human astrocytes, both P2Y2 and P2Y4 receptors trigger rapid mobilization of inositol trisphosphate-sensitive Ca^{2+} stores secondary to the activation of G_q-dependent, PI- PLC effector enzymes. The ability of natively expressed P2Y2 receptors to predominantly trigger PLC activation and Ca^{2+} mobilization in a very wide range of cell types (epithelial and nonepithelial) has been extensively characterized (e.g., Homolya et al., 1999, 2000). In contrast, with the exception of gerbil vestibular dark cell epithelium (Marcus and Scofield, 2001) there are few reported studies of signal transduction by natively expressed P2Y4 receptors. Although these nominally redundant P2Y receptor subtypes may use similar PLC- and Ca^{2+}-based pathways to regulate chloride transport in both airway and intestinal epithelia, P2Y2 and P2Y4 receptors may be differentially efficacious in activating alternative G protein-based signaling cascades used for the regulation of other integrated cell functions. The possible role of G_{12} or G_{13} based signaling pathways in mediating signaling by P2Y receptor subtypes has not been evaluated. Yuan et al. (2001) have demonstrated that certain G_q-coupled receptors can additionally initiate $G_{12/13}$ → rho → protein kinase D cascades in various cell types, including intestinal epithelial cells (Chiu and Rozengurt, 2001). In this regard, it is also germane to consider the nature of the Cl^- channels that mediate the increased short-circuit currents regulated by the P2Y receptors in airway versus jejunal epithelia. Robaye et al. (2003) stress that the Cl^- secretory response in control

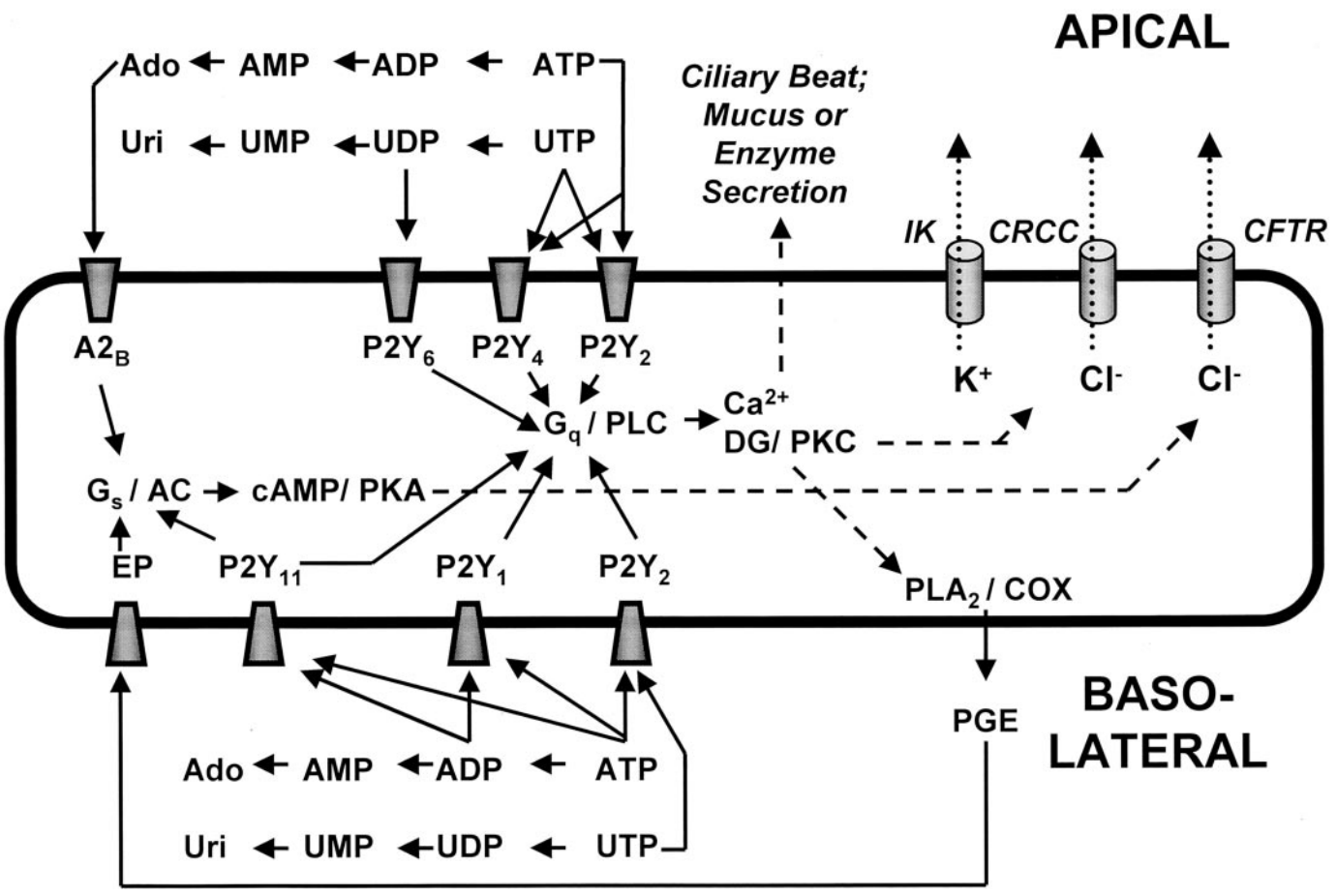

Fig. 1. P2Y receptor localization and signaling in a generalized epithelial cell. This compendium of observations from multiple epithelial cell types shows the known apical versus basolateral distribution of various P2Y receptor subtypes as well as the $A2_B$ receptor for adenosine (Ado) and the EP receptor for prostaglandin E. The cartoon also depicts the nucleotide selectivities of the P2Y subtypes, the ability of extracellular nucleotides to be serially catabolized by ecto-nucleotidases, and various integrated epithelial functions that can be targeted by P2Y receptor-induced signals. The latter include secretion, ciliary beating, and regulation of various ion channels such as K^+ channels (IK) in vestibular epithelial cells, Ca^{2+}-regulated Cl^- channels (CRCC) in airway and pancreatic epithelial cells, and CFTR Cl^- channels in airway, pancreatic, and intestinal epithelial cells.

murine jejuna is caused by activation of cystic fibrosis transmembrane regulator (CFTR) channels. Previous studies of CFTR-knockout mouse models have demonstrated that intestinal epithelia, unlike airway and pancreatic tissues, lack expression of alternative, non-CFTR Cl^- channels (Grubb and Gabriel, 1997; Lazarowski et al., 2001). In contrast, Cl^- secretion in the airways and other tissues (e.g., gallbladder and pancreas) can additionally be mediated by outwardly rectifying Cl^- channels that can be directly regulated by increased Ca^{2+} (Lazarowski et al., 2001; Wong and Ko, 2002). Although CFTR is characteristically regulated by cAMP→PKA pathways, activity of this Cl^- channel can also be modulated by PKC, PKG, and other protein kinases/phosphatases (Dahan et al., 2001). [It should be stressed that Robaye et al. (2003) ruled out the most obvious indirect loops of ATP/UTP → cAMP accumulation by performing the SCC experiments in the presence of indomethacin, to repress autocrine stimulation of G_s-coupled EP receptors and A2 receptor antagonists, to inhibit activation of G_s-coupled adenosine receptors). Thus, it is interesting to speculate that P2Y4 receptors may be more efficacious than P2Y2 receptors in activating atypical PKC or other kinase/phosphatase pathways that can be used for the regulation of CFTR; this may be an advantage in those epithelial tissues that express CFTR as the only, or principal, Cl^- secretory mechanism.

Another reason for the utilization of P2Y4 receptors versus P2Y2 receptors in the intestine may be possible differences in the rates and extents of receptor desensitization or down-regulation. For example, Brinson and Harden (2001) have defined major differences in the desensitization/internalization of P2Y4 receptors versus P2Y6 receptors when heterologously expressed in a common 1321N1 astrocyte background. Tissue-dependent differences in the activation or inactivation of particular P2Y receptor subtypes could affect the timing and duration of salt and fluid secretory responses to nucleotide agonists. In this regard, it is also worth considering the potential physiological or pathological sources of the extracellular ATP/UTP required to activate the apical P2Y4 receptors of the jejunum versus the P2Y2 receptors of the airways: nucleotide release from resident cells of the epithelia versus nonresident cells that periodically invade, or migrate to, the tissue. The resident cells in most epithelia can release endogenous ATP via nonlytic mechanisms in response to various mechanical stimuli, such as flow-induced shear stress and hypotonicity-induced swelling (Taylor et al., 1998; Homolya et al., 2000; Guyot and Hanrahan, 2002). These are obvious stimuli that can rapidly change in the gut depending on diet, frequency of eating, and infection with gastrointestinal pathogens. In the gut (but also in the airways), nonresident cells, such as pathogenic bacteria (Crane

et al., 2002) or invading leukocytes (Resnick et al., 1993), can also act as significant sources of extracellular nucleotides for the transactivation of the P2Y and/or adenosine receptors expressed in the resident epithelial cells. Whether the expression of particular P2Y receptor subtypes might be correlated with differences in the appearance or clearance of extracellular nucleotides within various epithelial tissues is an open and intriguing area.

As with most knockout mouse models that are largely "normal", this initial report from Robaye et al. (2003) raises a host of ancillary questions. Is there any significance regarding the X-linked chromosomal location of the murine P2Y4 receptor gene? Might the strong expression of P2Y4 receptor mRNA in the stomach and liver be indicative of other less appreciated roles for extracellular nucleotides in gastric secretion/motility or hepatic function? Might the selective apical expression of the P2Y4 receptor in the vestibular apparatus of other rodents (Sage and Marcus, 2002) suggest that the P2Y4-null mice may exhibit differences in spatial orientation or maintenance of equilibrium during rapid motions? This debut of the P2Y4 receptor-null mouse provides a portal to these and other new areas of investigation.

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