

P2Y Receptors:

Mediators of Extracellular Nucleotide Actions

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The notion that adenine and uridine nucleotides could function as extracellular signaling molecules was met with considerable resistance when it was proposed more than twenty years ago. However, this concept has been widely embraced in the last decade with the identification of two families of nucleotide-activated cell surface receptors: the P2X and the P2Y receptors. The P2X receptors are ligand-gated ion channel receptors and will not be discussed here. The P2Y receptors belong to the G-protein coupled receptor superfamily and were found to be expressed in virtually all cells and tissue types. P2Y receptors mediate an astounding array of biological functions including platelet aggregation, immune regulation, regulation of ion fluxes in airway epithelia and smooth muscle cell proliferation.

P2Y: The Receptors

Eight different functional mammalian P2Y receptors have been identified at this time (see Table 1) and their tissue distribution and signaling properties have been studied. As mentioned above, the P2Y receptors belong to the G-protein coupled receptor (GPCR) superfamily and hence present the signature molecular topology of an extracellular N-terminus, an intracellular C-terminus and seven transmembrane spanning motifs. Differing from other GPCR families, the P2Y show little sequence homology thus explaining the marked differences in ligand selectivity and specificity of G-protein (i.e. $G_{q/11}$ vs. G_i).

P2Y1, P2Y2, P2Y4 and P2Y6 bind to $G_{q/11}$ and hence activate phospholipase C (PLC) and mobilize intracellular Ca^{2+} while P2Y12, P2Y13 and P2Y14 bind to G_i , inhibit adenylate cyclase and reduce cAMP intracellular levels. P2Y11 is unique in that it can bind both $G_{q/11}$ and G_i and therefore couple ligand binding to both intracellular signaling pathways.^{1,2}

In addition to these signaling mechanisms, the P2Y receptors mediate their cellular effects by influencing other pathways such as inhibition of N-type voltage-gated Ca^{2+} channels (i.e. $Ca_v2.2$ channel) in neurons and endocrine cell lines, activation of G protein-gated inward rectifier K^+

channels (i.e. $K_{ir3.1}$ and $K_{ir3.2}$) in neurons and activation of Cl^- channels in airway epithelium.³

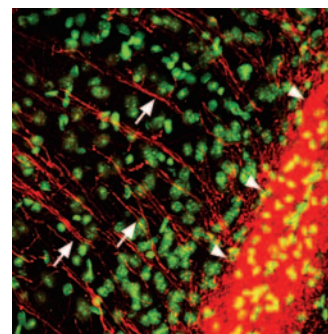
P2Y: The Ligands

The concept of adenine and uridine nucleotides as extracellular signaling molecules has been slow to take hold despite the fact that the involvement of adenine compounds in vasodilatation and platelet aggregation was described more than 70 and 30 years ago respectively.^{4,5} Today it is widely acknowledged that nucleotides are released from virtually every cell and influence a vast array of biological functions.

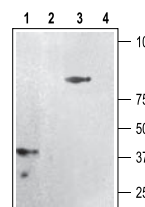
As demonstrated in Table 1, the different P2Y receptors mediate the actions of ATP, ADP, UTP, UDP and even UDP-glucose.

The mechanisms of the extracellular release of the different nucleotide compounds are still under intense scrutiny.⁶ In general, extracellular release of nucleotides from excitatory or secretory cells (i.e. neurons and pancreatic acinar cells) overlaps that of neurotransmitter release and therefore it is believed that nucleotides are packed in specialized granules such as synaptic vesicles and released by regulated exocytosis following the appropriate stimulus. The mode of nucleotide release from non-excitatory cells has proven more difficult to elucidate. In several tissue types such as airway epithelium, mechanical stimulation of

Expression of P2Y12 in Mouse Brain



Immunohistochemical staining of P2Y12 (red fluorescence) with Anti-P2Y12 antibody (#APR-012) and counterstaining with sytox green (green fluorescence). The region shown in this picture is the entorhinal cortex in mouse brain. The P2Y12 positive fibers (white arrows) run from the corpus callosum (white triangles) into the deep layers of the entorhinal cortex.



Western blotting of rat brain (1,2) membranes or human platelets (3,4):

1,2. Anti-P2Y12 antibody (#APR-012) (1:200).

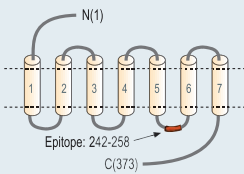
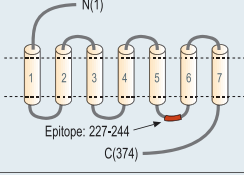
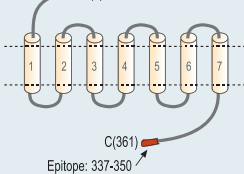
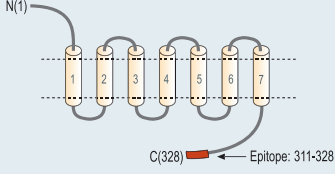
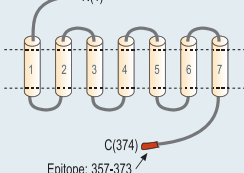
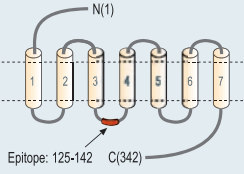
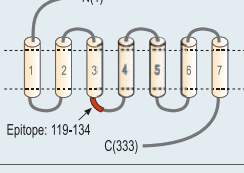
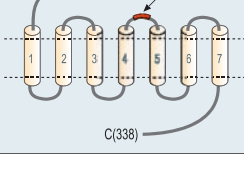
3,4. Anti-P2Y12 antibody, preincubated with the control peptide antigen.

the cells has been shown to induce nucleotide secretion while both passive and ligand-induced nucleotide release from several cell types, has been proposed.⁶

In addition, the extracellular nucleotide concentration is closely regulated by the action of several membrane-associated ectoenzymes such as the ecto-nucleotidase 5'-triphosphate

diphosphohydrolase (NTPDase1, also known as CD39) that selectively hydrolyzes nucleoside triphosphates into diphosphates.⁷

The P2Y Receptor Family

Receptor	Ligand	Signaling	Tissue Distribution	Antibodies	Epitopes
P2Y1	ADP	PLC↑, Ca ²⁺ ↑, PKC↑	Wide, including platelets, CNS, heart, skeletal muscle, GI tract.	Anti-P2Y1 (#APR-009)	
P2Y2	ATP/UTP	PLC↑, Ca ²⁺ ↑, PKC↑	Wide, including lung, heart, skeletal muscle, kidney, brain.	Anti-P2Y2 (#APR-010)	
P2Y4	UTP	PLC↑, Ca ²⁺ ↑, PKC↑	Placenta, lung, vascular smooth muscle (more widespread distribution in rodents).	Anti-P2Y4 (#APR-006)	
P2Y6	UDP	PLC↑, Ca ²⁺ ↑, PKC↑	Wide, including lung brain, heart, placenta, spleen, intestine.	Anti-P2Y6 (#APR-011)	
P2Y11	ATP	PLC↑, Ca ²⁺ ↑, PKC↑ and AC↓, cAMP↓	Spleen, intestine, dendritic cells	Anti-P2Y11 (#APR-015)	
P2Y12	ADP	AC↓, cAMP↓	Platelets, brain	Anti-P2Y12 (#APR-012)	
P2Y13	ADP	AC↓, cAMP↓	Brain, spleen	Anti-P2Y13 (#APR-017) (In preparation)	
P2Y14	UDP-glucose	AC↓, cAMP↓	Wide, including brain, heart, adipose tissue, placenta, intestine and hematopoietic cells.	Anti-P2Y14-extracellular (#APR-018)	

Abbreviations: PLC: Phospholipase C; PKC: Protein kinase C; AC: Adenylate cyclase; cAMP: cyclic AMP; CNS: central nervous system; GI Tract: gastrointestinal tract

P2Y: Functional Activity

As mentioned above, the functions of the different P2Y receptors are as varied as their tissue distribution (see Table 1) and detailed consideration of P2Y functions is beyond the scope of this review. A few examples of the involvement of P2Y receptors in physiological functions will be considered below.

As noted above, one of the earliest reported examples of extracellular nucleotide signaling has been their involvement in platelet aggregation. Platelets are blood cells whose role is to ensure blood vessel integrity and rapid cessation of bleeding following injury. When blood vessel injury occurs, platelets adhere to the sites of damage and form a monolayer of cells that cover the exposed tissue. Concomitantly they secrete several factors that initiate clot formation and recruit other platelets to the area. These prothrombotic factors include among others serotonin, thromboxane A₂ and ADP. It is now well established that extracellular ADP has a crucial role in platelet activation and that this function is mediated through the P2Y₁ and P2Y₁₂ receptors expressed in platelets.⁸ Based on this realization several antagonists to both receptors that show anti-thrombotic activities are being developed for clinical use.⁹

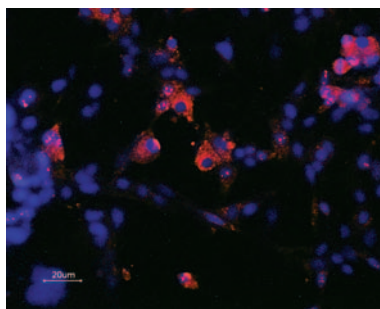
Another example in which P2Y receptor function is clinically relevant is in cystic fibrosis (CF) patients. CF is a genetic disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. CFTR encodes an epithelial membrane protein which functions as a cAMP-dependent Cl⁻ channel and a regulator of the epithelial Na⁺ channel (EnaC). A defective CFTR leads to abnormal fluid and solute transport across the airway epithelia and therefore to the formation of viscous, dehydrated mucus in airways, which produce progressive loss of ventilatory function and chronic pulmonary inflammation.

P2Y₂ is abundantly expressed in the apical membranes of several epithelial tissues including airway. It was shown that activation of the P2Y₂ receptor with ATP or UTP results in increased Cl⁻ efflux by way of activation of a Ca²⁺-dependent Cl⁻ channel that bypasses the CFTR channel. The activation of P2Y₂ receptors can therefore at least partially restore transepithelial water and salt secretion helping alleviate symptoms in CF models. Indeed, inhaled P2Y₂ agonists are being tested as a new generation treatment for CF patients.¹⁰

Finally, several P2Y receptors are expressed on cells of hematopoietic origin such as macrophages, dendritic cells and B lymphocytes and a number of roles in inflammation, regulation of the innate immune response and regulation of hematopoietic stem cells have been described.^{11,12}

Expression of P2Y14 in Differentiated

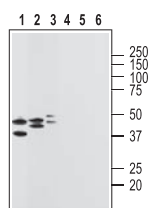
Rat PC12 cells



Immunocytochemical staining with Anti-P2Y14-extracellular antibody (#APR-018). Pheochromocytoma (PC12) cells differentiated with mNGF (50 ng/ml, #N-240) for 6 days and then stained with Anti-P2Y14-extracellular antibody. The red color represents the specific staining of P2Y14 receptors and the blue color represents the cell nucleus stained with DAPI.

Expression of P2Y14 in Rat Heart,

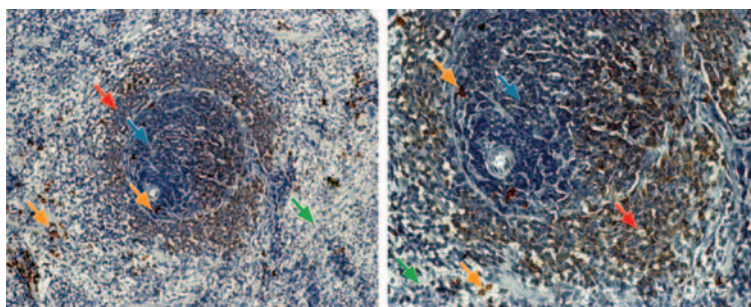
Brain and Lung



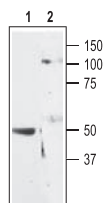
Western blotting of rat heart membranes (lanes 1,4), rat brain (lanes 2,5) and rat lung lysates (lanes 3,6):

1,2,3. Anti-P2Y14-extracellular antibody (#APR-018) (1:200).
4,5,6. Anti-P2Y14-extracellular antibody, preincubated with the control peptide antigen.

Expression of P2Y11 in Rat Spleen



Staining of P2Y11 receptor with Anti-P2Y11 antibody (#APR-015) in rat spleen. Secondary (activated) follicle in the spleen white pulp showing intense staining of Marginal Zone T lymphocytes (red arrow); note that cells in the red pulp (green arrow) and B lymphocytes in the germinal center (blue arrow) are not stained. Orange arrows show mononuclear cells with intense stain, probably non specific due to very high intrinsic peroxidase activity. DAB product is brown and the counterstain is Cresyl Violet. The two images are the same at different augmentation (Left pane 200x and right pane 400x).



Western blotting of human platelets lysates:

1. Anti-P2Y11 antibody (#APR-015) (1:200).
2. Anti-P2Y11 antibody, preincubated with the control peptide antigen.

References:

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Related Products

Compound	Product #
Antibodies	
Anti-P2Y1	APR-009
Anti-P2Y2	APR-010
Anti-P2Y4	APR-006
Anti-P2Y6	APR-011
Anti-P2Y11	APR-015
Anti-P2Y12	APR-012
Anti-P2Y14-extracellular	APR-018
Anti-EnaCβ	ASC-019
Anti-EnaCγ	ASC-011
Anti-CFTR	ACL-006