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Characterization of the UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family

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The cloning of a human G-protein-coupled receptor (GPCR) that specifically responds to UDP-glucose and related sugar-nucleotides has been reported recently. This receptor has important structural similarities to known members of the P2Y receptor family but also shows a distinctly different pharmacological response profile. Here, the IUPHAR Subcommittee for P2Y receptor nomenclature and classification review the current knowledge of this receptor and present their reasons for including this receptor in the P2Y receptor family as the $P2Y_{14}$ receptor.

UDP-glucose serves well-established biochemical roles in the synthesis of carbohydrates and protein glycosylation. However, it is far less appreciated that this nucleotide and other related sugar-nucleotides can exert pharmacological activity and thus receptor(s) for these molecules might exist. The recent cloning of a human GPCR (also known as GPR105 or KIAA0001) that specifically responds to UDP-glucose and related sugar-nucleotides [1] has important structural similarities to known members of the P2Y receptor family (which use simpler nucleotides as their agonists), but also shows a distinctly different pharmacological response profile. We discuss the pharmacological and signalling properties of this novel receptor and its potential role(s) in intercellular communication.

Subclassification of P2 nucleotide receptors

The role of extracellular adenine (ATP and ADP) and uracil (UTP and UDP) nucleotides as signalling molecules is phylogenetically ancient and universal in plants and animals. Receptors for these compounds – the P2 receptor family – are found on the surface of all animal tissues [2,3]. The existence of P2X and P2Y receptor subtypes was first suggested, on the basis of pharmacology, by Burnstock and Kennedy [4] in 1985. Following later studies of transduction mechanisms and cloning, in 1994 Abbracchio and Burnstock [5] proposed that P2 receptors should be subdivided into two families: (1) P2X ionotropic ligand-gated ion channel receptors; and (2) P2Y metabotropic G-protein-coupled receptors. This proposal has been adopted unanimously, with growing numbers of subtypes recognized since that time [3,6].

Current and putative P2Y receptors

The recognized members of the P2Y receptor family are the mammalian $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ receptors [7–10]. The missing numbers in the $P2Y_{1-n}$ sequence represent receptors cloned from non-mammalian vertebrates (whose mammalian orthologues have not yet been identified), or receptors that are currently under

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functional characterization. These and other potential members of the P2Y receptor family will be the subjects of future publications from the IUPHAR Subcommittee for P2Y receptor nomenclature and classification.

Alignment of the deduced amino acid sequences of the cloned P2Y receptors shows that human members of this family are 21–57% identical. When conservative substitutions are taken into consideration (i.e. where amino acid residues have been exchanged by other residues of similar structure or charge), the degree of relatedness among these receptors is much higher (36–69%). Pharmacologically, P2Y receptors can be subdivided into the adenine-nucleotide-preferring receptors mainly responding to ADP and ATP (human and rodent $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$, and human $P2Y_{11}$), the uracil-nucleotide-preferring receptors (human $P2Y_4$ and $P2Y_6$) responding to either UTP or UDP, and receptors of mixed selectivity (human and rodent $P2Y_2$ and rodent $P2Y_4$) [7–12].

Structural motifs involved in P2Y receptor activity

From a phylogenetic and structural point of view, two distinct P2Y receptor subgroups with a relatively high level of structural divergence have been identified [10,13]: the first subgroup encompasses $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors and the second subgroup includes $P2Y_{12}$ and $P2Y_{13}$ receptors (Fig. 1). Site-directed mutagenesis of P2Y receptors, to probe for regions of agonist–receptor interactions, has suggested that four amino acid residues of the transmembrane (TM) regions TM6 and TM7 might be important for agonist potency and specificity [12,14,15]. In particular, all cloned P2Y receptors share the TM6 H-X-X-R/K motif, which is crucial for agonist activity (Fig. 2). For $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors, a Y-Q/K-X-X-R motif in TM7 is also considered to participate in ligand binding. In the long-sought platelet $P2Y_{12}$ receptor [9] and the recently identified $P2Y_{13}$ receptor [10,11], this defining motif is substituted with K-E-X-X-L (Fig. 2), which suggests that these receptors possess a different mode of agonist binding.

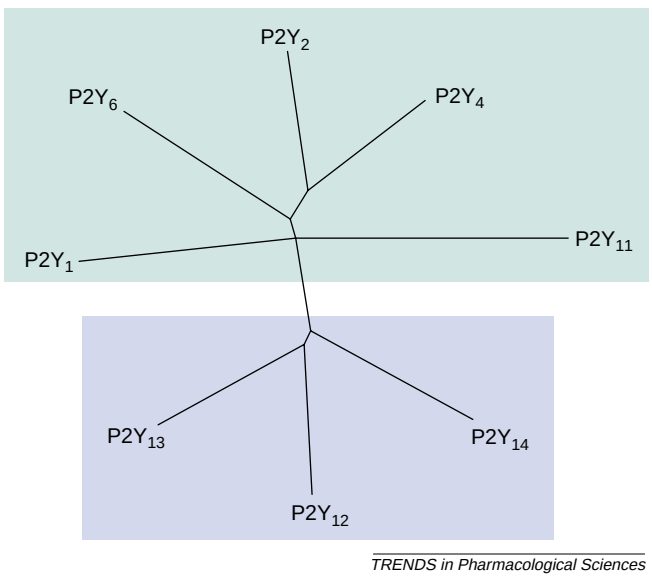

Fig. 1. A phylogenetic tree (dendrogram) showing the relationships among the current members of the P2Y receptor family (human $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ receptors) and the human UDP-glucose receptor (here indicated as the $P2Y_{14}$ receptor). The P2Y receptors can be divided into two subgroups shown with green and blue backgrounds. Sequences were aligned using CLUSTALX and the tree was built using the TREEVIEW software.

	TM6	TM7
$hP2Y_1$	AVSYIPFHVMKTMN	TYQVTRGLASLNSCVDP
$hP2Y_2$	ALCFLPFHVTRTLY	AYKVTRPLASANSCLDP
$hP2Y_4$	AVCFVPFHITRTIY	VYKVTRPLASANSCLDP
$hP2Y_6$	AISFLPFHITKTAY	AYKGTRPFASANSVLDP
$hP2Y_{11}$	ASSYVPYHIMRVLN	GYQVMRGLMPLAFCVHP
$hP2Y_{12}$	FICFVPFHFARIPY	VKESTLWLTSLNACLDP
$hP2Y_{13}$	FVCFAPFHFARVPY	AKETTLFLAATNICMDP
$hP2Y_{14}$	FVCFVPYHIARIPY	MKEFTLLLSAANVCLDP

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Fig. 2. Alignment of putative nucleotide binding motifs in transmembrane domain 6 (TM6) and TM7 of human (h) P2Y receptors. All receptor subtypes share in TM6 the presence of the H and R/K amino acid residues proposed to be crucial for receptor activity. In $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors, a Y-Q/K-X-X-R motif in TM7 has also been proposed to participate in ligand binding. In $P2Y_{12}$ and $P2Y_{13}$ receptors and in the UDP-glucose receptor (here indicated as the $P2Y_{14}$ receptor subtype), this motif is substituted with K-E-X-X-L. Crucial amino acids for nucleotide binding are highlighted in red. Sequences were aligned using CLUSTALX.

The final number of P2Y receptors is likely to exceed the cloned members listed above. This is based on the following considerations: (1) pathophysiological responses have been reported to be mediated by P2Y-like receptors characterized by pharmacological profiles that do not readily correspond with the known recombinant P2Y receptors [16,17]; and (2) a series of 'orphan' GPCRs (i.e. cloned receptors available in the public database for which a natural ligand has not been identified) share significant sequence identity with some P2Y receptors. Importantly, these orphan receptors also retain the amino acid motifs in TM6 and TM7 that are considered to be essential for P2Y receptor activation. In line with this hypothesis, one of these orphan receptors (formerly known as GPR86, GPR94 or SP174) is the $P2Y_{13}$ receptor [10,11]. Moreover, some of the cloned P2Y receptors cluster in the same regions of human chromosomes where several orphan GPCRs are also present. A cluster of seven related GPCRs, consisting of the $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors and four other receptors, has been found in human chromosome 3q24–3q25 [18]. Three of these genes (*GPR87*, *GPR91* and *H963*) still await identification; however, the fourth gene (*GPR105*) has been identified recently as the UDP-glucose receptor [1].

The UDP-glucose receptor

Similarities with known P2Y receptors

The UDP-glucose receptor was originally cloned from human myeloid cells (KIAA0001) and designated an orphan receptor (GPR105) [19]. This human receptor is most closely related in structure to the orphan GPCRs H963, GPR34 and EBI2; however, the thrombin and platelet-activating factor receptors represent the closest relatives with known functions. Sequence comparisons reveal that $P2Y_{1,2,4,6,11}$ receptors are also structurally related to the UDP-glucose receptor, and that they might share a recent common ancestor [1]. Alignment of the sequences of the UDP-glucose

receptor and human P2Y receptors reveals an overall identity of 18–45% (with highest identities in the regions corresponding to the TM1–TM7 domains) and further reveals the retention of the typical H-X-X-R and K-E-X-X-L motifs found in $P2Y_{12}$ and $P2Y_{13}$ receptors (Fig. 2). In the dendrogram of P2Y receptors shown in Fig. 1, the UDP-glucose receptor lies with the $P2Y_{12}$ and $P2Y_{13}$ receptors in the second main branch of the P2Y family.

The characteristic TM6 and TM7 amino acid motifs indicated above are also fully conserved in a previously cloned rat receptor (initially named VTR15–20 because it was isolated from the ventral tegmentum of rat) [20], whose complete sequence has been reported recently [21], as well as in the sequence of mouse GPR105 [21]. These receptors showing 80% and 83% amino acid identity, respectively, to the human UDP-glucose receptor [21], and indeed represent its rodent orthologues.

Pharmacological profile and coupling to G proteins

In recombinant systems that express human GPR105 or its rodent orthologues, the sugar-nucleotides UDP-glucose, UDP-galactose, UDP-glucuronic acid and UDP-*N*-acetyl-glucosamine exhibited agonist activity, with EC_{50} values in the 100–500 nM range [1,21]. No significant responses were detected with ATP, ADP, UTP, UDP, other nucleotides, dinucleotides, nucleosides, and other sugar-nucleotides, demonstrating a distinctly different pharmacological profile from that of the currently known P2Y receptors. Cell membranes expressing human GPR105 responded to UDP-glucose with increased binding of radiolabelled GTPγS – a characteristic indicator of G-protein activation [1]. Pertussis toxin completely abolished agonist responses, confirming the receptor to be coupled to G proteins of the $G_{i/o}$ class [18]. The transduction pathway(s) used by this receptor in native systems still remains to be defined.

Tissue distribution and potential functional roles

Widespread distribution of GPR105 has been observed in humans, with highest expression in placenta, adipose tissue, stomach and intestine, and moderate levels in the brain, spleen, lung and heart [1]. This expression pattern differs slightly from that reported in the rat for VTR15–20, with higher levels of expression in cells of haemopoietic origin (including kidney, liver, lung and spleen) and lower, yet significant, expression in discrete brain regions [20]. The recently reported tissue distribution of the mouse UDP-glucose receptor [21] is in broad agreement with the results obtained with the human receptor. Rat VTR15–20 has also been detected in several cell lines (including astrocytoma, neuronal and promyelocytic cells), and in rat primary microglia and astrocytes [20]. To confirm the potential importance of this receptor in glial cells, rat primary astrocytes have been shown recently to respond to UDP-glucose with increases of intracellular Ca^{2+} concentration [M. Fumagalli *et al.*, unpublished]. Interestingly, VTR15–20 has been reported to be regulated by immunological challenge. Following challenge with zymosan, a stimulator of macrophage phagocytosis, expression of VTR15–20 in rat primary microglia and astrocytes was significantly upregulated [20]. Acute *in vivo* lipopolysaccharide challenges in the rat also resulted in upregulation of VTR15–20 mRNA in discrete brain regions [20]. These data suggest that, at least in the rat, the UDP-glucose receptor might link the humoral and nervous systems to infection and inflammation.

One of the key elements in the definition of a membrane receptor-mediated signalling system is the demonstration of the release of the putative natural agonist into the extracellular space. Recently, using an enzymatic and high performance liquid chromatography (HPLC)-based methodology, the release of UDP-glucose in the lumen of primary cultures of bronchial epithelial cells from normal subjects and from patients with cystic fibrosis has been detected [22]. Under resting conditions, UDP-glucose levels were found in the 10–20 nM range. Similar levels have been detected in other cell lines including Calu-3 endothelial, 1321N1 astrocytoma, C6 glioma, Cos-7 and Chinese hamster ovary (CHO)-K1 cells.

Concluding remarks

UDP-glucose and UDP-galactose have long been thought to act exclusively as activated carriers of sugar moieties in the intermediary metabolism of carbohydrates. The data reviewed above showing the existence of a specific membrane receptor for UDP-glucose and its upregulation following immunological challenge requires a careful reappraisal of the physiological roles of these substances. Furthermore, based on: (1) sequence homology with cloned P2Y receptors; (2) the presence of key amino acids essential for receptor activation; (3) chromosomal colocalization with other P2Y receptors; (4) similarity of the cognate ligand to the natural ligands for P2Y receptors; and (5) identification of UDP-glucose in the extracellular space, the IUPHAR Subcommittee rename this receptor the $P2Y_{14}$ receptor.

The limited evidence on the potential pathophysiological roles of the $P2Y_{14}$ receptor needs to be confirmed by functional studies, preferably including the development of null alleles (i.e. knockout mice), anti-sense and RNA interference technology, and the development of small-molecule antagonists. However, the similarity of UDP-glucose and related molecules to the natural ligands for the P2Y receptors, which have established roles in intercellular communication, provides an exciting challenge for future research. It also encourages a search for new members of the $P2Y_{12-14}$ branch of this ubiquitous receptor family because the divergence of the two branches is so great that new members could not have been detected by homology screening based on the previously known P2Y receptors.

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Using genome-wide mapping in the mouse to identify genes that influence drug response

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Differential drug response is most often likely to be a complex trait, controlled by the combined influences of multiple genes and environmental influences. As a result of theoretical and technical limitations, to date, most clinically useful pharmacogenomic studies in humans have been limited to a small number of candidate genes that have a relatively major impact on drug response. Here, the problems involved in identifying genes that underlie drug response in humans are discussed and the power of mouse genetics as a tool for pharmacogenomic discovery is highlighted.

Pharmacogenomics is the study of how genetic inheritance influences response to drugs. A greater understanding of the genetic determinants of drug response has the potential to revolutionize the use of many medications. By increasing our ability to prospectively identify patients at risk for severe toxicity, or those likely to benefit from a particular treatment, pharmacogenomics promises to help us move towards the ultimate goal of individualized therapy.

There are many potential sources of pharmacogenomic variability in the human population. When a drug is given orally, it can be absorbed through the gastrointestinal tract, metabolized in the liver, delivered to its site of action by the systemic circulation, interact with its target, and be eliminated from the body through excretion into the bile and urine. Therefore, drug efficacy or toxicity can be modified by the interaction of the drug with numerous gene products. This can be illustrated by examples of genes from various functional categories that influence the outcome of drug treatment. For example, genes involved in drug transport [e.g. polymorphisms in the gene encoding P-glycoprotein 1 (*ABCB1*) and the plasma concentration of digoxin], genes involved in drug metabolism [e.g. polymorphisms in the gene encoding thiopurine S-methyltransferase (*TPMT*) and thiopurine toxicity] and genes encoding drug targets [e.g. polymorphisms in the gene encoding the β_2-adrenoceptor (*ADRB2*) and response to β-adrenoceptor agonists] all influence drug response [1].

Therefore, drug response is most often likely to be a complex (polygenic) trait, with multiple genes and environmental factors contributing with differing strengths to the overall phenotype. As such, the genetic analysis of drug response in humans is complicated by many factors. For

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