



Published in final edited form as:

Trends Pharmacol Sci. 2005 January ; 26(1): 8–9. doi:10.1016/j.tips.2004.10.010.

The recently deorphanized GPR80 (GPR99) proposed to be the P2Y₁₅ receptor is not a genuine P2Y receptor

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The P2Y receptor family currently encompasses eight members (P2Y_{1,2,4,6,11,12,13,14} receptors) that are activated by extracellular adenine and/or uracil nucleotides, or, in the case of the P2Y₁₄ receptor, by sugar-nucleotides, and are each characterized by the typical seven-transmembrane domain topology of G-protein-coupled receptors (GPCRs) [1]. For several years, it has been known that a series of ‘orphan’ GPCRs (i.e. cloned receptors available in the public database for which a natural ligand has not yet been identified) share significant sequence identity with the eight genuine P2Y receptors [1,2], and thus putatively represent novel members of the P2Y receptor family.

In March 2004, Inbe and colleagues reported that one of these orphan receptors [human GPR80 (also known as GPR99)], when stably transfected in HEK293 cells, could be selectively activated by both AMP and adenosine, and was thus renamed the P2Y₁₅ receptor as the latest member of the P2Y receptor family [3]. Identification of functional ligands for the expressed GPR80/GPR99/P2Y₁₅ receptor was based on Ca²⁺ mobilization and cAMP generation in response to a panel of extracellular ligands. Only AMP and adenosine induced

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a Ca²⁺ response in the transfected cells; both compounds did not induce such a response in HEK293 cells transfected with an identical vector construct. In the cAMP assay, both AMP and adenosine induced a response in the transfected cells. Non-transfected HEK293 cells similarly generated cAMP in response to adenosine (probably as a result of the stimulation of endogenously expressed adenosine receptors, as suggested by the authors themselves) but did not respond strongly to AMP [3]. Identification of GPR80/GPR99 as a receptor for adenosine and AMP was surprising because of the phylogenetic distance of the protein sequence of this receptor from the known receptors for adenosine, and because, in the first original study of GPR99 [4], this receptor failed to respond to AMP (or other nucleotides) in either the oocyte or the Chinese hamster ovary (CHO) cell expression system.

A few weeks following the report by Inbe *et al.*, He and colleagues reported that two 'orphan' GPCRs, anticipated to be nucleotide receptors [2] (i.e. GRP99 and GPR91), actually functioned as receptors for two citric acid cycle intermediates, α -ketoglutarate and succinate, respectively [5]. Receptor 'deorphanization' was achieved using aequorin and FLIPR (fluorimetric imaging plate reader) assays for intracellular Ca²⁺ mobilization in CHO cells, with EC₅₀ values of 69±11 μ M (aequorin assay) and 32±4 μ M (FLIPR assay) for α -ketoglutarate activation of human GPR80/GPR99. α -Ketoglutarate also activated mouse GPR80/GPR99. The close functional relationship between GPR80/GPR99 and GPR91 was confirmed following the discovery that only these two receptors, of ~300 GPCRs examined, contain four basic amino acid residues (i.e. Arg99, His103, Arg252 and Arg281) that are required to bind the dicarboxylate groups of α -ketoglutarate and succinate. In addition to demonstrating that, by acting as ligands for GPCRs, citric acid cycle intermediates have unexpected signalling functions beyond their traditional metabolic roles, these data are in contrast to the results of Inbe *et al.* described earlier and identify α -ketoglutarate as the physiological ligand for human GPR80/GPR99.

During the 4th International Symposium on Nucleosides and Nucleotides held recently in Chapel Hill, NC, USA (6–9 June, 2004), the members of the International Union of Pharmacology (IUPHAR) Subcommittee for P2Y receptor nomenclature and classification carefully examined the report by Inbe and colleagues [3] and the report by He and colleagues [5] in an attempt to establish whether or not to accept GRP80/GPR99 as the P2Y₁₅ receptor. Members raised strong reservations regarding the exclusive use of HEK293 cells in the study by Inbe *et al.* because this cell line is known to express several endogenous P2Y receptors in addition to the P1 adenosine receptors A_{2A} and A_{2B}, which might complicate the pharmacological characterization of novel receptors. Moreover, adenosine A₁ and P2Y₁ receptors have been shown previously to form heterodimers that enable the A₁ receptor to bind and respond to ADP [6], and thus it is possible that, at least in part, responses to AMP and adenosine in HEK293 cells expressing GPR80/GPR99 are influenced by a physical interaction between GPR80/GPR99 and endogenous P2Y or A_{2B} receptors, resulting in a novel pharmacological response profile. In addition, another study has shown that expression of GPR80/GPR99 in several cell lines (CHO, COS and another subclone of HEK293) did not result in responses to either adenosine or AMP [7]. Similarly, in COS-7 transfected cells, ATP, ADP, UTP, UDP and UDP-glucose failed to induce any response, thus confirming that GRP80/GPR99 is not a P2Y receptor [7]. Instead, and consistent with the report by He *et al.* [5], α -ketoglutarate promoted ³H-inositol phosphate accumulation in

CHO and HEK293 cells expressing GPR80/GPR99 [7]. It was thus concluded that the results of Inbe *et al.* were more likely to be due to high levels of expression of endogenous adenosine receptors in the clone of HEK293 cells expressing GPR80/GPR99, and/or to heterodimer formation (see also [8]) or another artefact.

Thus, the P2Y Receptor Nomenclature Subcommittee has decided that GPR80/GPR99 is not a P2Y receptor for adenosine, AMP or other nucleotides, but instead is activated by citric acid cycle intermediates. To avoid confusion in P2Y receptor nomenclature, the Subcommittee strongly recommends that the name of P2Y₁₅ should not be used in the future when referring to genuine functional receptors for extracellular adenine and uracil nucleotides. Furthermore, future members of the P2Y receptor series should use P2Y₁₆ as the next available name.

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