Letters

The recently deorphanized GPR80 (GPR99) proposed to be the $P2Y_{15}$ 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 $P2Y_{14}$ 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 $P2Y_{15}$ receptor as the latest member of the P2Y receptor family [3]. Identification of functional ligands for the expressed GPR80/GPR99/$P2Y_{15}$ receptor was based on Ca^{2+} mobilization and cAMP generation in response to a panel of extracellular ligands. Only AMP and adenosine induced a Ca^{2+} 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 Ca^{2+} mobilization in CHO cells, with EC_{50} values of 69 ± 11 μM (aequorin assay) and 32 ± 4 μM (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 $P2Y_{15}$ receptor. Members raised strong reservations regarding

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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 A_1 and $P2Y_1$ receptors have been shown previously to form heterodimers that enable the A_1 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 ^{3}H-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 $P2Y_{15}$ 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 $P2Y_{16}$ as the next available name.

References

1 Abbracchio, M.P. *et al.* (2003) Characterization of the UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family. *Trends Pharmacol. Sci.* 24, 52–55

2 Boeynaems, J-M. *et al.* (2003) P2Y receptors: new subtypes, new functions. *Drug Dev. Res.* 59, 30–35

3 Inbe, H. *et al.* (2004) Identification and characterization of a cell-surface receptor, $P2Y_{15}$, for AMP and adenosine. *J. Biol. Chem.* 279, 19790–19799

4 Wittenberger, T. *et al.* (2002) GPR99, a new G protein-coupled receptor with homology to a new subgroup of nucleotide receptors. *BMC Genomics* 3, 17–23

5 He, W. *et al.* (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 429, 188–936

6 Yoshioka, K. *et al.* (2001) Heteromeric association creates a P2Y-like adenosine receptor. *Proc. Natl Acad. Sci. U. S. A.* 98, 7617–7622

7 Qi, A-D. *et al.* GPR80/99, proposed to be the $P2Y_{15}$ receptor activated by adenosine and AMP, is not a P2Y receptor. *Purinergic Signalling* (in press)

8 Suarez Gonzales, N. *et al.* The fate of P2Y-related orphan receptors: GPR80/99 and GPR91 are receptors for dicarboxylic acids. *Purinergic Signalling* (in press)

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Erratum

Erratum: The pharmacology of cough

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In the article 'The pharmacology of cough' by Reynolds *et al.*, which was published in the November 2004 issue of *TiPS*, there were three errors. In Box 1, sibenadet was incorrectly cited (in the text and table) as a dopamine D2 receptor and β_2-adrenoceptor antagonist. It is in fact a D2 receptor and β_2-adrenoceptor agonist. In the section 'GABA receptors' on p. 574, baclofen was incorrectly cited as a $GABA_B$ receptor antagonist. It is in fact a $GABA_B$ receptor agonist. In addition, Ref. 36 should have been cited as Adcock, J.J. *et al.* (2003) RSD931, a novel anti-tussive agent acting on airway sensory nerves. *Br. J. Pharmacol.* 138, 407–416. The authors and *TiPS* apologise to readers for these errors.

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