ABSTRACT

Identification and characterization of G protein-coupled nucleotide and nucleotide-like receptors P2Y13 and GPR17

Purinergic receptors are divided into three subfamilies, P1 (adenosine), P2 (nucleotide) and P0 (adenine) receptors. P2 receptors comprise ligand-gated ion channels - shortly LGICs, receptors $P2X_{1\text{-}7}$, and class A, δ-branch GPCRs termed $P2Y_{1,2,3,6,12\text{-}14}$. The $P2Y_{13}$ receptor is physiologically activated by ADP and coupled to G_i whose activation results in an inhibition of adenylate cyclase. $P2Y_{13}$ receptor activation has been reported to be neuroprotective. $P2Y_{13}$ receptors also play a role in lipoprotein metabolism and cholesterol transport, making the receptor an interesting target for the potential treatment of atherosclerosis.

The nucleotide-like orphan receptor GPR17, whose endogenous agonist remains still unknown, is also G_i-coupled. GPR17 appears to be involved in demyelinating diseases such as multiple sclerosis, and some inflammatory diseases.

The development of potent, selective ligands, agonists and antagonists, for both $P2Y_{13}$ and GPR17, is required to study their physiological and pathophysiological roles and for validating the receptors as future drug targets.

In the present study, we aim to find out, if the approach via β-arrestin assay employing an enzyme complementation is suitable for a closer characterization of the human $P2Y_{13}$ and the human GPR17 receptor. Moreover, we want to study whether this approach would be useful for compound screening, identifying activators or inhibitors of each of those two receptors.

The human GPR17 containing a β-galactosidase fragment was expressed in Chinese hamster ovary cells (CHO) that express β-arrestin containing the second complementary fragment of β-galactosidase (obtained from DiscoveRx). This recombinant cell line is used for test compound screening. GPR17 activation leads to β-arrestin recruitment which results in enzyme complementation and allows β-galactosidase-dependent chemiluminiscence measurement.

Our goal is to screen for agonists and antagonists of GPR17 and to obtain an analogous cell line for the human $P2Y_{13}$ receptor, which will allow the determination of $P2Y_{13}$ receptor activation by measuring luminescence. A β-arrestin assay will be established with an outlook to determine the potency of agonists and antagonists.

Key words: $P2Y_{13}$, GPR17, β-arrestin assay, compound screening