Comment on “Identification of Novel G Protein–Coupled Receptor 143 Ligands as Pharmacologic Tools for Investigating X-Linked Ocular Albinism”

We read with interest the recent article in IOVS by De Filippo and colleagues1 “Identification of Novel G Protein–Coupled Receptor 143 Ligands as Pharmacologic Tools for Investigating X-Linked Ocular Albinism” as we have extensive experience in this area of research. We are writing to point out a critical design flaw with the study that complicates interpretation of the data presented in the article.

De Filippo and colleagues study GPR143, a G-protein–coupled receptor (GPCR) in commercial culture medium containing 215 μM tyrosine. Importantly, we have shown previously that tyrosine at high concentrations binds to, activates, and promotes internalization of GPR143. Therefore, custom cell culture media needs to be made to study the biology of GPR143. Before we demonstrated this phenomenon, several reports also mischaracterized GPR143 as an intracellular receptor due to their use of commercial cell culture media, some of which have 457.5 μM tyrosine (as is found in standard Dulbecco’s modified eagle medium [DMEM]). When cells are maintained in custom media containing 1 μM tyrosine, GPR143 has the standard cellular distribution of other GPCRs: it is found at the plasma membrane due to cytoplasmic loop mutations we have observed in mice with ataxia and a benign phenotype and at the endosomal compartment.1,5 In human RPE in situ, devoid of potential cell culture artifacts, we also demonstrate that GPR143 is on the plasma membrane, making it like every other GPCR studied to date.4,5 During these studies, we discovered that the endogenous ligand for GPR143 is L-DOPA, having a higher affinity for GPR143 than closely related tyrosine.2,5 We also showed that L-DOPA added to media causes an increase in intracellular calcium in cultured RPE, studying both the endogenous and overexpressed recombinant receptor.2 We observed no alteration in cAMP and no sensitivity to pertussis toxin. We concluded that the endogenous ligand for GPR143 is L-DOPA, which is present at a sufficiently high concentration in the subretinal space (4.82 μM) to activate GPR143 during development.6 The ocular L-DOPA is produced by the RPE during melanogenesis, is largely retained by the RPE, and is not converted to dopamine.6,7 As a comparison, the dopamine receptors (D1-5) exhibit a range of affinities for dopamine from 4 nM to 7 μM,8 consistent with what we observed for GPR143's natural ligand. L-DOPA activation of GPR143 recruits β-arrestin, controls growth factor release,9 regulates exosome release in situ,10 and influences recruitment of myocilin to the endosomal compartment.11

In several other cell types (including Chinese hamster ovary [CHO] transfected to overexpress GPR143), we observed low background activity of GPR143 in media containing 1 μM tyrosine and a response to L-DOPA that is immediate and robust (Figure). In contrast, De Filippo and colleagues1 report a very high level of constitutive activity of GPR143 and lack of response to L-DOPA. This is not surprising given the 215 μM tyrosine in the media used in their study. Tyrosine, dopamine, and L-DOPA all compete for the same GPR143 binding site.2 Also not unexpected is that De Filippo and colleagues were only able to screen for antagonists in their assay given the continuous activation of receptor (likely by tyrosine in the commercial medium chosen). Instead of simply making custom media, the authors use a mutant form of GPR143 that remains at the plasma membrane due to cytoplasmic loop mutations that inhibit GPR143 endocytosis. For their assay to be useful to screen for agonists, we think they simply need to adjust their culture media, reducing the tyrosine concentration, which would abrogate the need to use the mutant receptor with unknown agonist activity.

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Acknowledgments

Supported by National Eye Institute grant EY026544-02 (to BSM).

Disclosure: B.S. McKay, P; R.M. Lynch, None; W.D. Stamer, None

References


Citation: *Invest Ophthalmol Vis Sci*. 2017;58:4733–4734. doi:10.1167/iovs.17-22660