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 colleagues. “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. Thus, 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 GPCR 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 overexpressed recombinant receptor.2 We observed no alteration in Ca2+ and no sensitivity to pertussis toxin. We maintained in custom media containing 1 μM tyrosine. Tyrosine, dopamine, and L-DOPA all compete for the same GRP143 binding site.2 We also showed that L-DOPA activation of GPR143 recruits b-arrestin, controls neurotrophic factor release,2,3 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 colleagues 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 agonists 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.

Brian S. McKay1
Ronald M. Lynch2
W. Daniel Stamer3,4

1Department of Ophthalmology and Vision Science, University of Arizona, Tucson, Arizona, United States; 2Department of Physiology, University of Arizona, Tucson, Arizona, United States; 3Department of Ophthalmology, Duke University, Durham, North Carolina, United States; and the 4Department of Biomedical Engineering, Duke University, Durham, North Carolina, United States.

E-mail: bsmckay@eyes.arizona.edu.

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