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[1] "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 213 μM tyrosine. Importantly, we have shown previously that tyrosine at high concentrations binds to, activates, and promotes internalization of GPR143.[2] 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 having 457.5 μM tyrosine (as is found in standard Dulbecco's modified eagle medium [DMEM]).[3] When cells are maintained in custom media containing 1 μM tyrosine, GPR143 has the standard cellular distribution of other GPCRs: it is found in the synthetic pathways, plasma membrane, and endosomal compartment.[2,4,5] 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] 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 neurotrophic factor release,[2,9] 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 colleagues[1] report a very high level of constitutive activity of GPR143 and lack of response to L-DOPA. This is not surprising given the 213 μM tyrosine in the media used in their study. Tyrosine, dopamine, and L-DOPA all compete for the same GRP143 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.

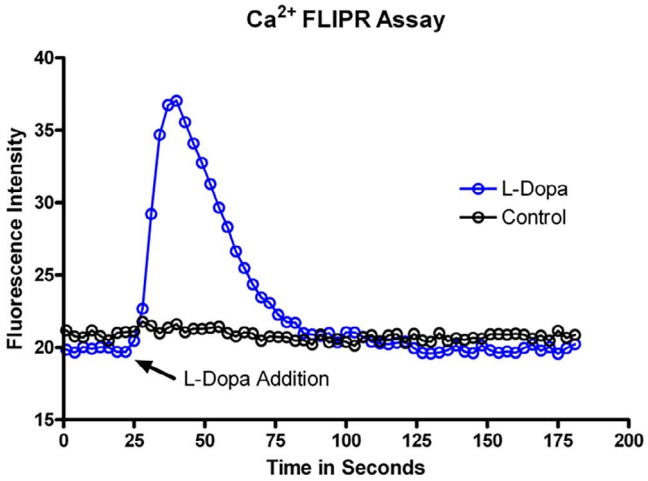

Figure. FLIPR fluorometric imaging analysis of intracellular calcium in CHO cells expressing recombinant GPR143. CHO cells were transfected to express human GPR143 and maintained in tyrosine-free DMEM supplemented with 10% dialyzed fetal bovine serum (devoid of free amino acids) and 1 μM tyrosine. Cells were incubated as described for the FLIPR Calcium Assay kit, and fluorescence was measured using a Flex Station 3 (Molecular Devices, Sunnyvale, CA, USA). After a stable baseline measurement was achieved, L-DOPA was added to a final concentration of 1.0 μM. Control cells were maintained in normal medium without a ligand and illustrate the stable baseline in the absence of an activating ligand.

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