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DOI: 10.1002/anie.201600957

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Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Link to publication on Research at Birmingham portal

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Download date: 04. Oct. 2023
Allosteric Optical Control of a Class B G-Protein-Coupled Receptor

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Abstract: Allosteric regulation promises to open up new therapeutic avenues by increasing drug specificity at G-protein-coupled receptors (GPCRs). However, drug discovery efforts are at present hampered by an inability to precisely control the allosteric site. Herein, we describe the design, synthesis, and testing of PhotoETP, a light-activated positive allosteric modulator of the glucagon-like peptide-1 receptor (GLP-1R), a class B GPCR involved in the maintenance of glucose homeostasis in humans. PhotoETP potentiates Ca$^{2+}$, cAMP, and insulin responses to glucagon-like peptide-1 and its metabolites following illumination of cells with blue light. PhotoETP thus provides a blueprint for the production of small-molecule class B GPCR allosteric photoswitches, and may represent a useful tool for understanding positive cooperativity at the GLP-1R.

The incretin hormone glucagon-like peptide-1 (GLP-1) is released from enteroeocrine L-cells in the intestine, from where it binds cognate receptors to promote the survival of pancreatic beta cells, insulin release, and weight loss. For these reasons, incretin mimetics based on GLP-1 have become widely-prescribed drugs for the restoration of normal glucose levels in type 2 diabetes (T2D), a socio-economically costly syndrome affecting almost 400 million individuals worldwide.

The glucagon-like peptide 1 receptor (GLP-1R) is a class B G-protein-coupled receptor (GPCR) that is primarily coupled to adenylate cyclase activity and 3',5'-cyclic adenosine monophosphate (cAMP) accumulation, as well as intracellular Ca$^{2+}$ fluxes. Recently, an allosteric site has been described for this receptor that allows fine modulation of orthosteric ligand binding. The ligand-dependent allosteric activator 4-(3-(benzoyloxy)phenyl)-2-(ethylsulfanyl)-6-(trifluoromethyl)pyrimidine (BETP) potentiates Ca$^{2+}$ mobilization in response to GLP-1(7-36)NH$_2$, the active amidated form of GLP-1. By contrast, BETP amplifies cAMP generation in response to GLP-1(9-36)NH$_2$, a metabolite and weak partial GLP-1R agonist. Such interactions are therapeutically desirable, since drugs that target the GLP-1R allosteric site may improve receptor specificity, thereby reducing side effects. However, their investigation is at present hindered by a lack of specific research tools for the fine control of allosterism and receptor movement. Photopharmacology is well-suited to this task, since it relies on the properties of light to precisely deliver drug activity in space and time.

Herein, we describe the development and testing of PhotoETP, a light-activated positive allosteric modulator that allows optical control of GLP-1R signaling and insulin secretion by using blue light (Figure 1A).

We set out to confer photoswitching on the GLP-1R allosteric site by subjecting BETP to our “azologization” strategy (Figure 1B; see also Figure S1 in the Supporting Information). By coupling commercially available chloropyrimidine 1 and boronic acid 2 under Suzuki–Miyaura conditions, bisaryl thioether 3 was obtained in a yield of 95%. After oxidizing the sulfur atom with mCPBA to its sulfone counterpart 4 in a yield of 90%, it was exchanged in an aromatic substitution with ethyl sulfide to give ethyl thioether 5 in a yield of 55%. Subsequent oxidation with one equivalent of mCPBA gave access to sulfoxide 6 (96%), which was deprotected with TFA before undergoing Mills reaction with...
Characterization of PhotoETP. a) Isomerization of PhotoETP between its trans- and cis-states with blue light or UV irradiation, respectively. b) UV/Vis spectra of PhotoETP in DMSO following illumination at λ = 440 nm (blue), λ = 330 nm (grey), or under dark conditions (black). c) Robust photoswitching between trans- and cis-PhotoETP induced with λ = 440 nm and λ = 330 nm, respectively. d) LC–MS trace of PhotoETP in the dark (black) and after exposure to UV light (λ = 350 nm; grey).

The extent of photoswitching is similar to that recently reported for an allosteric modulator of the metabotropic glutamate receptor mGluR5, a class C GPCR. The effect of PhotoETP on cell viability was determined in islets by using necrosis and apoptosis assays in the dark. At the concentration used throughout the present study, PhotoETP did not induce significant necrosis (Figure 3B) or apoptosis (Figure 3C), as measured using propidium iodide incorporation and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), respectively. By contrast, treatment with staurosporine or thapsigargin positive controls resulted in large increases in necrotic and apoptotic indices (Figure 3B,C). Furthermore, levels of cleaved caspase-3, an enzyme involved in the proteolytic cleavage of critical intracellular effectors including poly (ADP-ribose) polymerase, were unaffected by incubation with PhotoETP (Figure S2).

Next, photoswitching of intracellular Ca\(^{2+}\) dynamics was assessed by using PhotoETP directly in beta cells residing within intact islets of Langerhans. Whereas cis-PhotoETP (λ = 350 nm) showed little effect, the trans isomer (λ = 440 nm) potentiated GLP-1(7-36)NH\(_2\)–induced increases in Ca\(^{2+}\) levels (Figure 3D–G), as previously described for cAMP. The latter could be abolished by using either low glucose (Figure S3A,B) or the specific GLP-1R antagonist exendin 9–39 (Figure S3C,D). In all cases, results in islets were replicated in MIN6 beta cells subjected to high-throughput Ca\(^{2+}\) screens (Figure 3H–J). When using batch-incubated islets, trans-PhotoETP potently amplified GLP-1(9-36)NH\(_2\)–induced insu...
PhotoETP was almost 20% cis-enriched under benchtop conditions (1H NMR spectrum; see the Supporting Information), whereas the UV/Vis spectra revealed a more pronounced \( \pi-\pi^* \) band in the dark (see Figure 2B). This finding can be explained by consulting a model of the glucagon receptor mutant F345C bound to BETP, where a twisted conformation of the benzylether is found to be the lowest energy state. Such a conformation may also be adopted by cis-PhotoETP owing to its higher affinity for covalent binding. However, in contrast to BETP, which can reorganize its molecular shape in response to orthosteric ligand binding, PhotoETP would remain trapped in its cis state until illumination to induce trans-isomerization. Although the exact isomer ratio at the receptor is difficult to determine empirically, such properties may nonetheless afford fine control over photoswitching, with dark conditions, 440 nm illumination, and 350 nm illumination leading to graded Ca\(^{2+}\) responses (Figure S4). Further studies using rigid E- and Z-stilbene bioisosteres of PhotoETP will be required to better delineate the mechanisms involved.

The data presented herein outline a straightforward synthetic strategy for the production of a blue-light-activated positive allosteric modulator, which enables photoscontrol of GLP-1R activity through a feedforward loop encompassing the orthosteric site (Figure 1A). Although similar “allo-witches” have been described for ionotropic and metabotropic mGlRs,\(^{12,13}\) this is the first demonstration of their use in a therapeutically relevant class B GPCR. Using a combination of Ca\(^{2+}\), cAMP, and insulin assays in CHO-GLP-1R and MIN6 cells, as well as islets of Langerhans, we were able to show that PhotoETP allows photoswitching of responses to GLP-1(7-36)NH\(_2\) and its less active breakdown product, GLP-1(9-36)NH\(_2\), with similar potency to native BETP. Notably, PhotoETP displays unusual behavior in cells, where it shows an enriched cis content when interacting with its target in the dark. Indeed, the more active trans isomer has to be photochemically induced by irradiation with blue light. As a result of these properties, PhotoETP together with the recently described signal-biased GLP-1R photoswitch LirAzo,\(^{14}\) may enable the precise dissection of allosteric–orthosteric cooperativity, molecular movement, and binding.

Both BETP and PhotoETP were more effective at potentiating GLP-1(9-36)NH\(_2\)-induced compared to GLP-1(7-36)NH\(_2\)-induced insulin secretion. This suggests that cAMP rather than Ca\(^{2+}\) is the primary driver of the “incretin effect”, and is consistent with previous results obtained using LirAzo.\(^{14}\) Intriguingly, optical control of insulin release could only be observed in GLP-1(9-36)NH\(_2\) and PhotoETP-treated islets, where blue light provoked a two-fold higher response than UV illumination. Although the exact reasons for this remain unknown, it may reflect an inability to detect relatively small isomer-induced differences in intracellular Ca\(^{2+}\) versus cAMP concentration at the level of secretion in islets.

BETP was susceptible to UV-A-induced but not white-light-induced reactions, thus making it a poor control for photoswitching purposes (see Figure S5–S8). In contrast, PhotoETP was remarkably robust. This protective effect...
stems from the azobenzene unit, which preferentially harvests UV-A photons with its π–π* band to undergo isomerization. In other words, by installing an azobenzene moiety onto BETP, side reactions can be quenched and the resulting molecule stabilized. Nevertheless, the UV-A-induced rearrangement of BETP to its sulfenic ester counterpart via a Meisenheimer complex, and the accompanying transformation, is in itself an interesting finding (Figure S5,S6). Although related rearrangements of sulfoxides have been reported,[13] sulfenic esters have not been isolated as products owing to the low (UV-C) wavelengths used in these experiments. Such rearrangements are relevant for drug activity, as best exemplified by acid-activation of the irreversible proton-pump inhibitor omeprazole (Prilosec).[16]

In summary, we showcase PhotoETP, a light-activated modulator for allosteric optical control of GLP-1R function, and highlight the requirement to run parallel control experiments with benchmark drugs in photopharmacology. Photo-ETP, or optimized derivatives thereof, may be useful in drug-discovery programs aimed at unraveling the complexity of allosterism and class B GPCR signaling.

Acknowledgments

J.B. was supported by an EFSD Albert Renold Young Scientist Fellowship and a Studienstiftung des deutschen Volkes PhD studentship. N.R.J. was supported by a Diabetes UK RW and JM Collins Studentship. H.M.B. was supported by a Bavarian Grant for Studies. B.J.L. was funded by an MRC Clinical Research Training Fellowship. S.R.B. is funded by grants from the MRC, BBSRC, NIHR, an Integrative Mammalian Biology (IMB) Capacity Building Award, an FP7-HEALTH-2009–241592 EuroCHIP grant and is supported by the NIHR Imperial Biomedical Research Centre Funding Scheme. G.A.R. was supported by Wellcome Trust Senior Investigator (WT098424/AIA), MRC Programme (MR/J000304/1), Diabetes UK Project Grant (11/0004210) and Royal Society Wolfson Research Merit Awards. D.J.H. was supported by Diabetes UK R&D Lawrence (12/0004431), EFSD/Novo Nordisk Rising Star and Birmingham Fellowships, as well as an MRC project grant (MR/N00275X/1). D.J.H. and G.A.R. were supported by Imperial Confidence in Concept (ICiC) Grants. D.T. was supported by an Advanced Grant from the European Research Council (268795). We thank Dr. Peter Mayer (LMU) for X-ray data collection and solving structures and Dr. Jun-ichi Miyazaki for providing MIN6 cells.

Keywords: allosteric regulation · beta cells · GLP-1 receptor · photopharmacology · type 2 diabetes

How to cite: Angew. Chem. Int. Ed. 2016, 55, 5865–5868
Angew. Chem. 2016, 128, 5961–5965


Received: January 27, 2016
Published online: April 5, 2016