Allosteric Optical Control of a Class B G-Protein-Coupled Receptor

Johannes Broichhagen, Natalie R. Johnston, Yorrick von Ohlen, Helena Meyer-Berg, Ben J. Jones, Stephen R. Bloom, Guy A. Rutter, Dirk Trauner,* and David J. Hodson*

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 enteroendocrine L-cells in the intestine,[3] from where it binds cognate receptors to promote the survival of pancreatic beta cells, insulin release, and weight loss.[2] 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),[3] a socio-economically costly syndrome affecting almost 400 million individuals worldwide.[4]

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,[5] as well as intracellular Ca\(^{2+}\) fluxes.[6] Recently, an allosteric site has been described for this receptor that allows fine modulation of orthosteric ligand binding.[7] 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\(_2,[7,8]\) the active amidated form of GLP-1. By contrast, BETP amplifies cAMP generation in response to GLP-1(9-36)NH\(_2,[7,8]\) 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.[7-9] 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.[10]

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[11] (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...
The effect of trans-cis isomerization of PhotoETP. a) Isomerization of PhotoETP. This allows Ca\(^{2+}\) and cAMP responses to GLP-1 and its inactive metabolites to be potentiated following illumination with UV or blue light. b) Six-step synthetic pathway for the production of PhotoETP. c) Crystallization of PhotoETP as its methoxy counterpart (7) from MeOH. d) Crystal structures for PhotoETP congener 7 (CCDC 1420305 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre) and its precursor bisaryl thioether 3 (CCDC 1420306 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre).

Figure 1. Design and synthesis of PhotoETP. a) An azobenzene unit is installed on the positive allosteric modulator BETP to produce PhotoETP. This allows Ca\(^{2+}\) and cAMP responses to GLP-1 and its inactive metabolites to be potentiated following illumination with UV or blue light. b) Six-step synthetic pathway for the production of PhotoETP. c) Crystallization of PhotoETP as its methoxy counterpart (7) from MeOH. d) Crystal structures for PhotoETP congener 7 (CCDC 1420305 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre) and its precursor bisaryl thioether 3 (CCDC 1420306 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre).

The UV/Vis spectrum of PhotoETP under illumination at \(\lambda = 440\) nm (trans, blue) and \(\lambda = 330\) nm (cis, gray) demonstrated the presence of wavelength-dependent switching (Figure 2A), as expected for a meta-azobenzene system (Figure 2B). Photoswitching could be repeated over several cycles with reasonably fast kinetics (\(\tau_{\text{off}} = 204.2 \pm 7.3\) s; \(\tau_{\text{on}} = 54.5 \pm 2.9\) s) and without obvious fatigue (Figure 2C), and the cis and trans isomers were separated by LC–MS analysis (Figure 2D). Together, these features of PhotoETP provide a basis for the allosteric photocyclin of GLP-1R activity.

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 (\(\lambda = 350\) nm) showed little effect, the trans isomer (\(\lambda = 440\) 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 extendin 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-
Intriguingly, optical control of insulin release could increases in intact PhotoEXTP and its less active breakdown product, was co-applied at 10 nM or GLP-1(7-36)NH2 in the presence of 8–2016 <trans>100 nM or GLP-1(7-36)NH2</trans> 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)NH2 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 Ca2+ 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,[15] 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.

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