PHOTOLEG

Dr. David Barber and Prof. Dirk Trauner (LMU Munich)

1. INTRODUCTION: Photopharmacology

The accurate control of biological function using an external stimulus is a challenging endeavour. However, recent advances in the field have revolutionised the way biological systems can be controlled, greatly progressing our understanding of complex cellular processes. The field of optogenetics is one such example.[1] By harnessing the power of light a variety of important ion channels and G-protein coupled receptors (GPCRs) can now be efficiently controlled with excellent spatial and temporal precision. One drawback of this method is that viral vectors have to be used to introduce the light controlled receptors into the organism of study. Photopharmacology aims to solve this issue by using small and freely diffusible photoswitches that can 'turn on' and 'turn off' biological targets when illuminated with different wavelengths of light (Figure 1).[2]

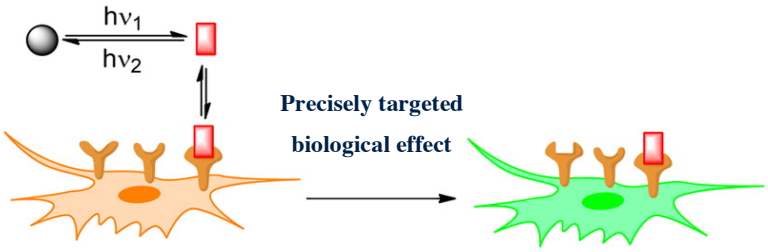

Figure 1. Concept of photopharmacology. The 'turned off' drug molecule (black circle) is converted to the 'turned on' drug molecule (red rectangle) using the spatial and temporal precision of light. Figure adapted from reference 2.

In our design concept, pharmacologically active molecules are converted to light controllable drugs by the incorporation of an azobenzene photoswitch. This can result in optical tools that can accurately and selectively control a range of pharmacological targets when exposed to different wavelengths of light. Further advancement of this concept could be used to create photopharmaceuticals that prevent undesired side effects and off-target interactions. The use of light as the controlling stimulus also provides numerous opportunities for therapeutic applications as it is relatively non-invasive and can be controlled with unrivalled temporal and spatial precision.[3]

2. OBJECTIVES OF THE PROJECT

Several photochromic agonists for ionotropic glutamate receptors (iGluRs) have already been reported.[4] However, these photoswitches 'turn on' iGluRs in the dark and require a light stimulus to be 'turned off'. This significantly decreases the suitability of these photoswitches for therapeutic applications, as long periods of light irradiation are required. In addition, the wavelength of the light used to control the photoswitch is often in the harmful UV region. Therefore, the ***main objective*** of this work was to develop new photoswitchable ligands for iGluRs that 'turn off' iGluRs in the dark and are then 'turned on' when irradiated with light. We also sought to develop photoswitches that respond to red-shifted wavelengths of light, removing the need to use high energy UV light. It is expected that success in these endeavours will ultimately allow for the development of new and innovative methods to treat degenerative brain diseases and restore vision by reactivating unresponsive neural networks in the brain and central nervous system.

3. RESULTS

We have designed and synthesised a family of photochromic antagonists of AMPA receptors (a sub-type of iGluRs) by incorporating a molecular photoswitch into the chemical structure of known AMPA receptor antagonists.[5] By analysing the structure activity relationships of the known antagonists, we recognised that an azobenzene could be

introduced in the 6-position of the quinoxaline-diketone core (Figure 2, highlighted in red). We also decided to use the antagonist **MPQX** as a template for our photoswitch design as it is a selective AMPA receptor antagonist and the phosphonic acid side chain aids its solubility in aqueous solutions.

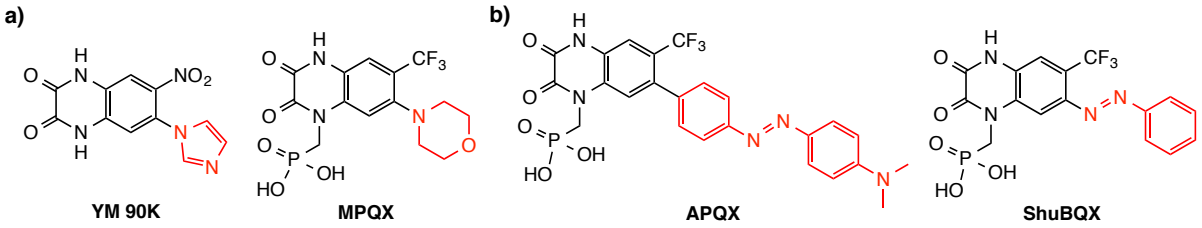

Figure 2. a) Molecular structures of the known AMPA receptor antagonists **YM 90K** and **MPQX**. b) Molecular structures of our photochromic AMPA receptor antagonists **APQX** and **ShuBQX**.

The photoswitch **ShuBQX** was identified as an excellent photoswitchable antagonist of AMPA receptors using patch-clamp electrophysiology of HEK293T cells expressing AMPA receptors. **ShuBQX** efficiently 'turns off' AMPA receptors in the dark and under illumination with long wavelength visible light. Upon illumination with blue light (440 nm or 460 nm) and in the presence of the AMPA receptor agonist glutamate, **ShuBQX** is deactivated, leading to the 'turning on' of the AMPA receptors (Figure 3a). **ShuBQX** was also shown to optically control native AMPA receptors in excitable cells. Using an acute mouse brain slice preparation and whole cell patch-clamp electrophysiology, **ShuBQX** (10 μM) and glutamate (100 μM) were locally applied to a hippocampal CA1 neuron and the induced action potential firing could be effectively controlled by switching between blue (460 nm) and orange (620 nm) light (Figure 3b). The NMDA-receptor antagonist AP-5 (50 μM) was also added locally to ensure that this effect was not due to NMDA receptors.

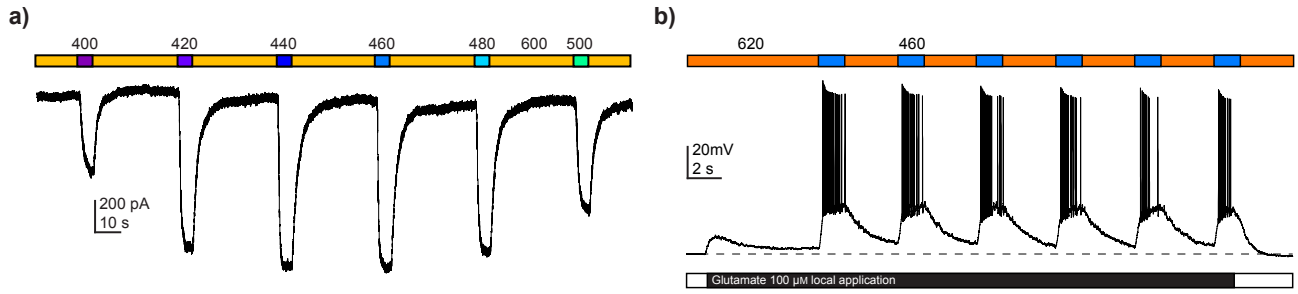

Figure 3. a) Whole cell patch-clamp experiment of **ShuBQX** (10 μM) and glutamate (300 μM) using HEK293T cells transfected with AMPA receptors. b) Optical control of action potential firing in hippocampal CA1 neurons using **ShuBQX** (10 μM) in the presence of glutamate (100 μM) and AP-5 (50 μM).

In summary, we have developed a photochromic antagonist that permits the precise optical control of AMPA receptors. Our photoswitch, **ShuBQX**, 'turns off' AMPA receptors in the dark and illumination with blue light 'turns on' AMPA receptors. **ShuBQX** expands the photopharmacology of iGluRs and demonstrates that potent photoswitchable antagonists can be developed. We envision that **ShuBQX** will become an important tool for studying the function of AMPA receptors *in vivo*, possibly leading to the development of new treatments for degenerative brain diseases and furthering efforts to restore vision.

1) L. Fenno, O. Yizhar, K. Deisseroth, *Annu Rev Neurosci.* **2011**, *34*, 389–412; 2) W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2014**, *136*, 2178–2191; 3) T. Fehrentz, M. Schönberger, D. Trauner, *Angew. Chem. Int. Ed.* **2011**, *50*, 12156–12182; 4) P. Stawski, M. Sumser, D. Trauner, *Angew. Chem. Int. Ed.* **2012**, *51*, 5748–5751; 5) D. Catarzi, V. Colotta, F. Varano, *Med. Res. Rev.* **2007**, *27*, 239–278.