A Dual Ca^{2+}/DAG Sensor Reports on Ligand Efficacy: Validation for the M3ACh Receptor

Alonso Cañizal M. C.[1,2], Winkler C.[1,2], Ziegler N.[1,2], Hoffmann C.[1,2]

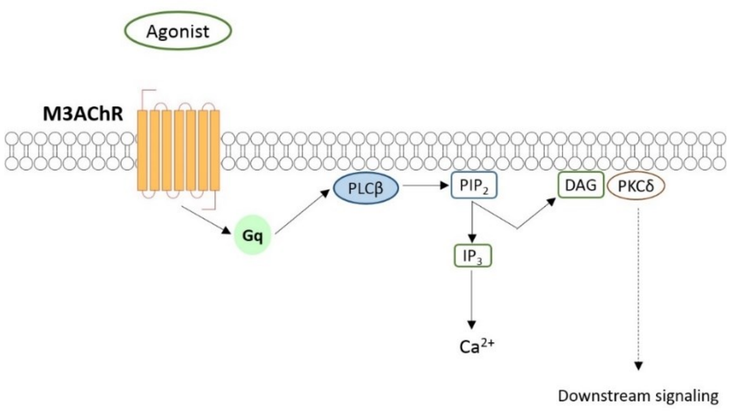
[1]Rudolf-Virchow-Zentrum, Bio-Imaging-Center - Würzburg, Germany
[2]University of Würzburg, Institut für Pharmakologie und Toxikologie - Würzburg, Germany

TAKE-HOME MESSAGE

A dual calcium/DAG sensor allows to observe the PLC signaling pathway specifically induced by the activation of the Muscarinic Acetylcholine Receptor 3 and produces a precise and differential response that correlates with the structural changes produced in the receptor upon its activation.

INTRODUCTION

Many GPCR activate the heterotrimeric protein Gq, which leads to the subsequent activation of the phospholipase C (PLC) pathway, producing an increase of calcium due to its release from the intracellular stores and the translocation of the protein kinase C (PKC) to the membrane for binding diacylglycerol (DAG).



We have validated a fluorescent probe that allows the simultaneous detection of DAG and Ca^{2+} in real time. Using this sensor, we study the specific activation of the M3ACh Receptor, a Gq-coupled GPCR that up-regulates PLC activity.

METHODS

System: two sensors fused in frame with a 2A peptide sequence in between:

1. DAG sensor: cpGFP fused to the C domains of PKC. Green signal decreases due its translocation to the membrane.
2. Ca^{2+} sensor: R-GECO. Red signal increases due to the release of Ca^{2+}

- HEK293 cells were co-transfected with the dual sensor and a CFP-tagged M3AChR.
- Using confocal microscopy, we tested 5 agonists at saturating ligand concentrations: acetylcholine, carbachol, oxotremorine M, oxotremorine and pilocarpine.
- FRET analysis with the 5 ligands: M3AChR sensor with the specific FIAsH binding sequence (CCPGCC) inserted within the N-terminal domain of the 3rd iloop and CFP fused to the receptor C-terminus.

RESULTS

1. Validation of the dual sensor in M3ACh Receptor

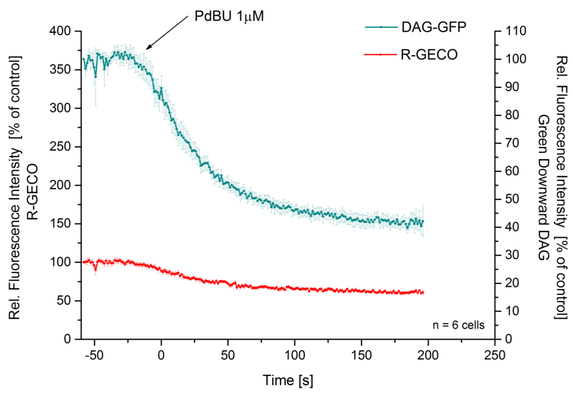


Fig. 1. PdBU is an activator of the PKC that produces a decrease of GFP signal without affecting calcium levels

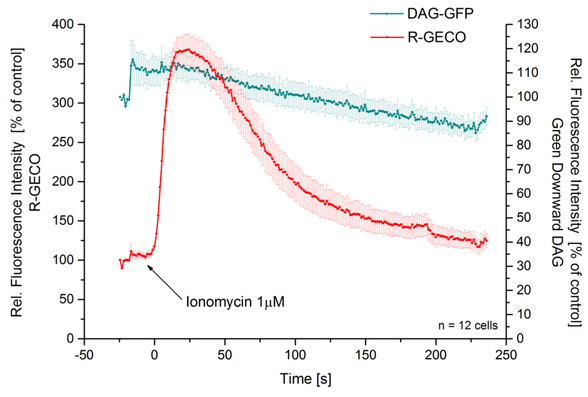


Fig. 2. Ionomycin is an ionophore that produces an increase of calcium without affecting DAG levels

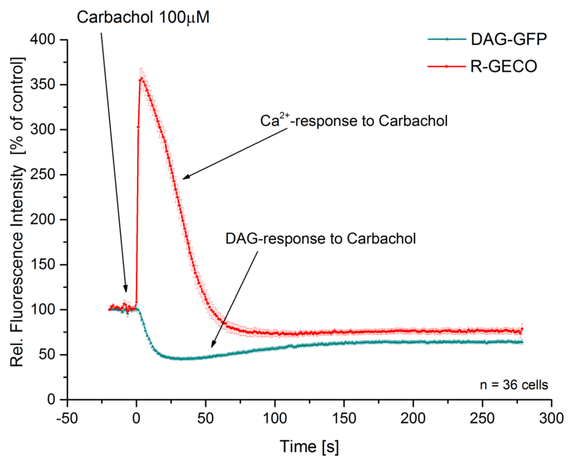


Fig. 3. Carbachol is a nonspecific agonist that binds M3AChR and activates PLC pathway, producing a DAG dependent signal and an increase in intracellular calcium levels.

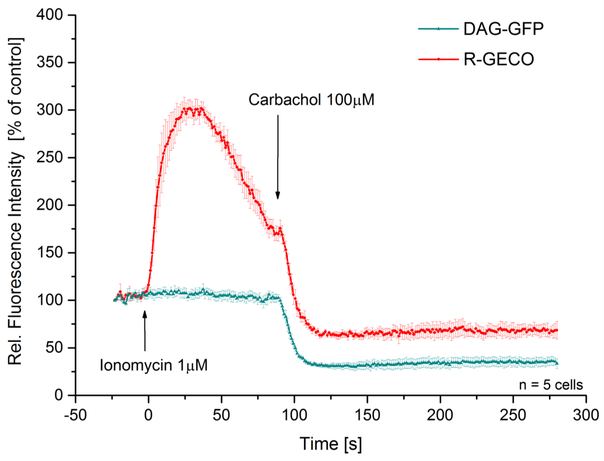


Fig. 4. The addition of Carbachol after Ionomycin produces an immediate decrease of the DAG dependent green signal.

2. The dual sensor allows to study the efficacy of different ligands over M3AChR activation

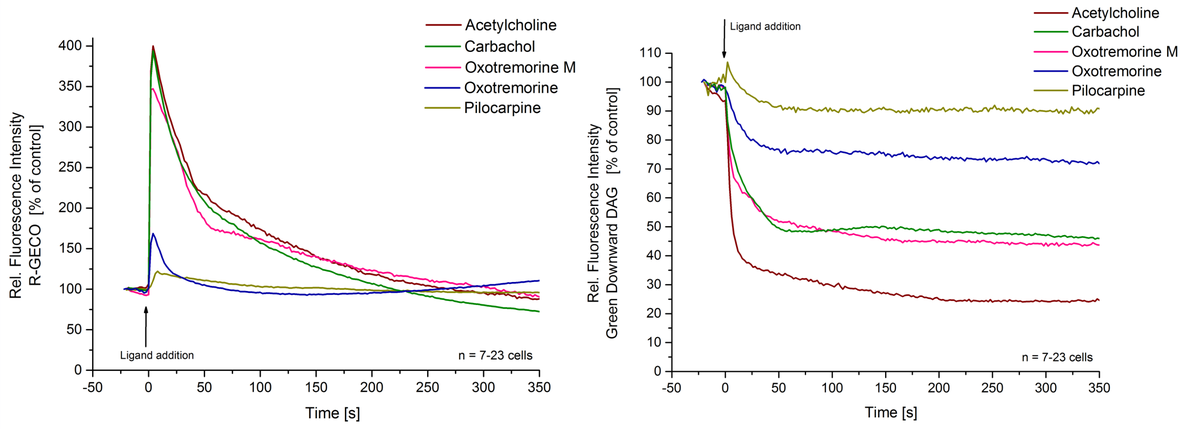


Fig. 5. All ligands produce a release of calcium and a DAG sensor response, which translates into an increase in R-GECO signal (left) and a decrease in GFP signal (right).

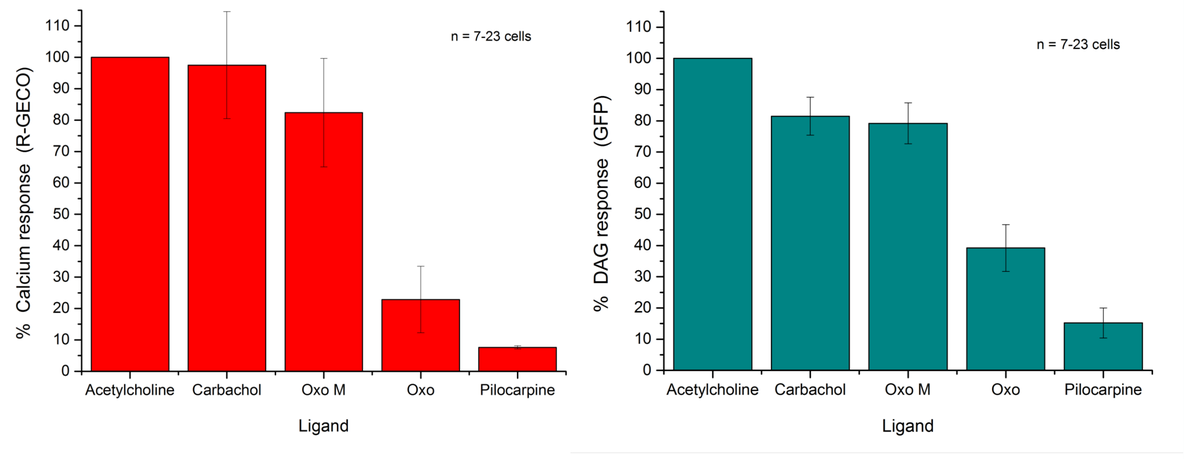


Fig. 6. Percentage of observed receptor response produced by the different ligands and expressed in relation to Acetylcholine response, as full agonist of M3AChR.

3. Conformational changes produced in M3AChR after the activation with different ligands

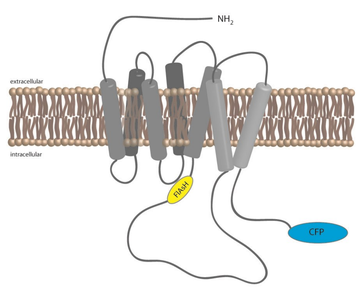
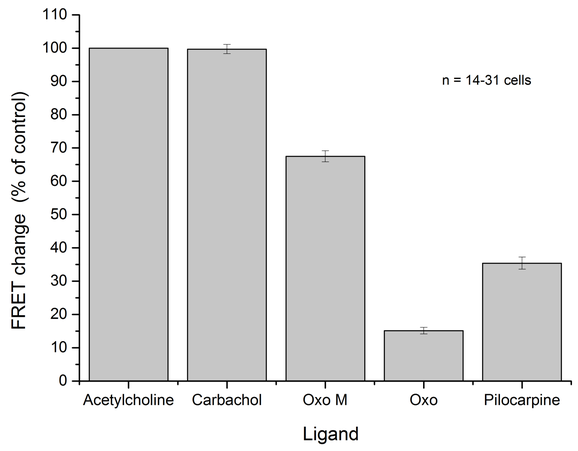


Fig. 7. Percentage of FRET change produced by the agonists with reference to Acetylcholine.

Activation of M3AChR by the ligands produced distinct conformational changes in the receptor.



4. The conformational change produced in M3AChR correlates with its activity

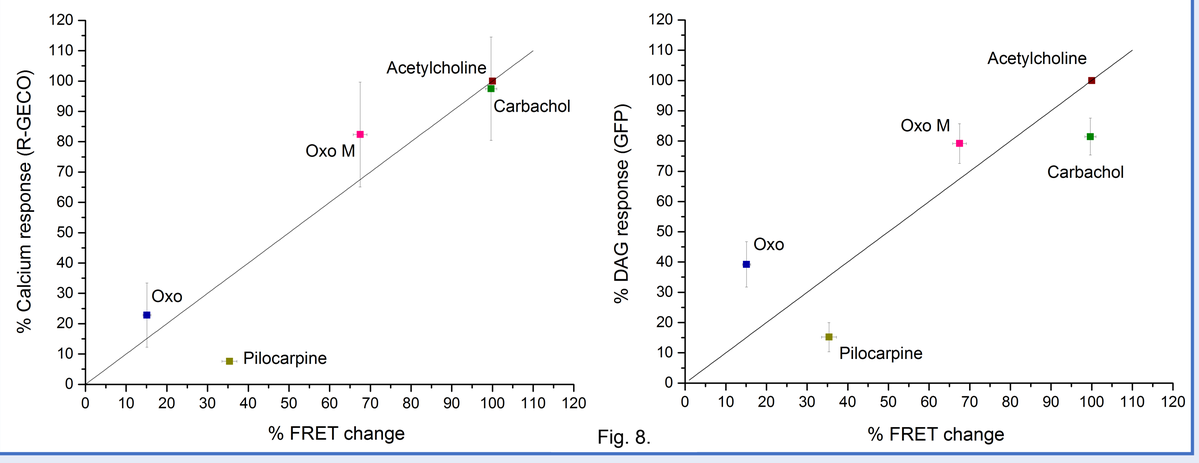


Fig. 8.

Contact:

consuelo.alonso_canizal@uni-wuerzburg.de