Interactions between G-protein Coupled Receptors and Ligand Gated Ion Channels

Reporting

Project Information

GPCR-LGIC COUPLING

Grant agreement ID: 268336

Status
Closed project

Start date
1 October 2010

End date
30 September 2014

Funded under
FP7-PEOPLE

Overall budget
€ 100 000

EU contribution
€ 100 000

Coordinated by
MIDDLE EAST TECHNICAL UNIVERSITY
Turkey

Final Report Summary - GPCR-LGIC COUPLING (Interactions between G-protein Coupled Receptors and Ligand Gated Ion Channels)

Recent studies demonstrate that many G-protein coupled receptors (GPCRs) form homo- or heterodimers. Adenosine A2A-Dopamine D2 receptor interaction is one of the examples for GPCR heterodimerization. Both receptors bear critical roles in physiological processes. Adenosine A2A receptor has functions in neurotransmission, cardiovascular system and immune response. On the other hand, dopamine receptors are the key point of dopaminergic system, which control the regulation of memory, attention, food intake, endocrine regulation, psychomotor activity and positive reinforcement. Dopamine D2R and adenosine A2AR have been shown to interact in striatum and modulate dopaminergic activity of which deregulation could cause neurological disorders such as Parkinson’s disease and schizophrenia. In addition to GPCR dimerization, GPCR-Ion channel interaction is an important regulator of synaptic activity. One of the most prominent interactions between ion channels and GPCRs is NMDAR-D2R heteromerization.

NMDA (N-methyl D-aspartate) Receptor is a ligand and voltage gated ion channel and involve in many processes such as synaptic plasticity, memory formation, behavioral responses and cell survival. Dopamine D2R and NMDA receptor interactions are important in many processes such as learning, memory, motivation, cognition and attention. Due to their participation in such critical processes, significant alterations in their interaction contribute directly to some neurological disorders such as schizophrenia.

The purpose of this study is to detect the physical interactions between A2A, D2R and NMDAR by using Fluorescence Resonance Energy Transfer (FRET) technique. For this purpose we tag these receptors with EGFP (enhanced green fluorescent protein) and mCherry (a red fluorescent protein) fluorophore in live N2a cell line and by using a laser scanning confocal microscope image the tagged receptors under various conditions. In addition interactions detected by using Biomolecular Fluorescence Complementation (BiFC) method. For this purpose, A2A and D2R receptors were tagged with split EGFP parts. Results obtained from these studies could be used to understand and explain molecular mechanisms of some neurophysiological events and disorders based on these receptors. Moreover, the fluorescence based live cell model could be used to detect effects of potential anti-psychotic drugs on the interactions between these receptors.

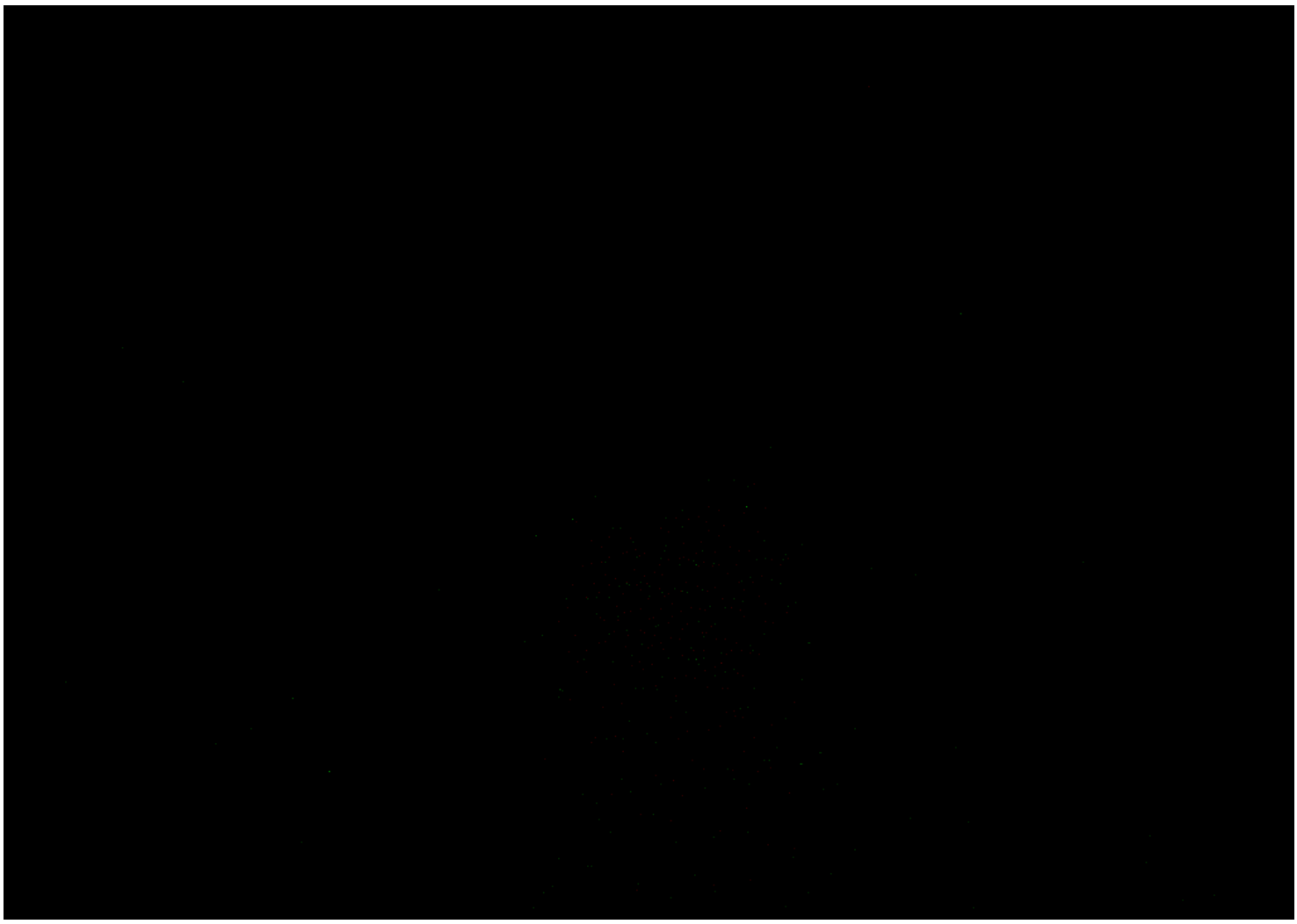
In the context of this study, functionality of tagged A2A and D2R receptors were tested with luminescent cAMP measurements. FRET studies that were carried by functional A2A and D2R receptors showed that A2A and D2R heterodimerization occurs mostly on the cell membrane. Details of the homo- and hetero dimerization, location and distance of the donor and acceptor fluorophores were discussed in the second periodic report, under the “Work Progress and Achievements during the period” section.

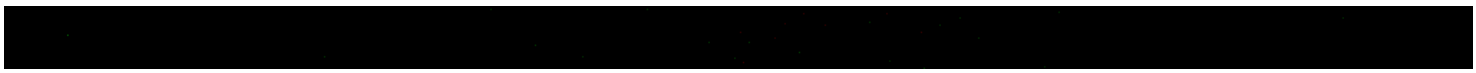
Imaging of EGFP and mCherry tagged NMDAR showed that NR1 subunit of NMDAR can be individually found in the cell whereas tagged NR2B subunit can only be seen in the presence of NR1 subunit 24 to 36 hour after transfection. Truncation of NR1 subunit after N-terminal domain enabled us to see N-terminal domain of NR1 is enough to prevent premature degradation of NR2B subunits in live cells. In this study, interaction of A2AR with NMDAR has been shown with 8.30 % FRET efficiency on average, which is less than that of A2AR homodimerization or heterodimerization with D2Rs. Test of triple interaction using combination of FRET and BiFC produced very week signal which was not possible to analyze. This result can be explained by several hypothesis; the most straight forward explanation is the triple interaction does not occur in live cells under the experimental conditions. Although both A2A-NMDA and D2R-NMDA interactions were shown, in literature and in this study our results suggests A2A-D2R heterodimer does not interact with NMDA receptors. Second possible explanation is the triple interaction exists but the detection limit of FRET-BiFC is not enough to detect the low level of triple interaction. Currently we do not have a method to test this hypothesis we are working on the improvements of the detection limit of the technique in live cells. Third possible explanation is the triple interaction exists but the placement of the split EGFP and mCherry is not suitable to obtain a FRET signal. Although weak, this possibility of fluorophore location causing low or undetectable FRET signal can be corrected by changing the location of the tags. Detection of heterodimerization between all possible combinations (A2A-D2R, A2A-NMDA, D2R-NMDA) indicates localization of the tags are proper for detection of protein-protein interactions. However in order to be through we are currently trying different tagging locations in these receptors.

Besides the detection of receptor interaction in live cells the project also aimed to test effects of possible drugs on these interactions. For this purpose we used FRET method to test effects of A2AR and D2R agonists on dimerization. While no significant effect was detected on observed dimerization concluded from average FRET efficiencies for whole cells, careful analysis of the A2AR FRET distribution upon agonist (CGS-21680) treatment a significant increase in the 10-20 % FRET efficiency pixels, suggested a change in receptor population in this group. According to FRET images, this group of receptor dimers occur mostly in the intracellular regions of the cell suggesting either increased synthesis and accumulation of receptor dimers in ER and Golgi or activation of the downregulation pathways upon over stimulation and accumulation of receptor dimers in endocytic vesicles. In order to comment on these hypothesis further experiments using organel markers and/or conditions preventing protein synthesis and endocytosis should be tested.

As a conclusion, techniques established and optimized during this project are suitable to test protein-protein interactions in live cells. Effect of drugs, dopamine and adenosine agonists and/or antagonists on dimerization can be studied by the optimized imaging techniques. Besides, this project and the results

obtained initiated 2 new grants on protein-protein interactions and imaging. Optimization of advanced fluorescent microscopy techniques, establishment of a new imaging laboratory, and training of four MSc. student directly involved in the project work and six additional MSc. students who were trained in the laboratory established partly by this grant has been achieved through out this Marie Curie Reintegration grant.





final1-3da2aegfpd2rmcherry0.gif

Last update: 12 May 2015
Record number: 163133