BRET² assay using the FDSS/µCELL: monitoring cell surface-receptor internalization

Introduction

After the publication of application note No. 23 describing BRET¹ technology[1] and based on a study published in 2011[2], Pierre Fabre Research Institute recently implemented the more sensitive BRET² technology to the FDSS/µCell system for monitoring the internalization of the human dopaminergic D2s- receptor (short splice form), a prototypic G protein-coupled receptor (GPCR). Internalization and endocytosis of GPCRs are dynamic processes that prevent from acute overstimulation. The inner cell-surface-located Kras GTPase protein can be used as a general inert marker for monitoring the presence or absence of a receptor at the plasma membrane. Upon receptor activation triggering its internalization, we could monitor distance changes between the receptor and the GTPase protein, and observed a BRET decrease between the receptor and the Kras.

Experimental procedure

Rluc8 tagged-D2s receptor (C-terminal) and GFP²-tagged GTPase Kras (N-terminal) were transiently transfected into CHO cells. We additionally coexpressed β-arrestin2 and GRK2 proteins. 48 hours after transfection, cells were washed twice with PBS plus glucose 1g/l, detached with PBS plus 3 mM EDTA and resuspended in PBS plus glucose 1g/l. A Dc protein measurement assay (Bio-Rad) was performed according to the manufacturer's protocol.

For BRET monitoring, cells were then distributed (70 µg protein equivalent per well) in white and non-treated clear-bottom 96-well microplates (Costar, ref. #3632) and incubated during 60 minutes at 37 °C in the presence or absence of various ligands before substrate addition. BRET between Rluc8 and GFP² was measured immediately after the addition of the Rluc8 substrate coelenterazine 400a (5 µM final) (Interchim, ref. #BB839B).

BRET² signals were collected using the FDSS/µCELL (Hamamatsu Photonics), allowing to integrate successive signals detected sequentially in the 387-447 nm (Rluc8) and in the 489-532 nm (GFP²) windows by using appropriate filters. Time of integration of 0.5 second was used for measuring photons emitted by Rluc8 and GFP² (Additional parameters: sensitivity 8; samples number 4). BRET² signals were obtained by calculating the ratio of the fluorescence intensity of GFP over luminescence intensity of Rluc8.

Results

Monitoring GPCR internalization at the plasma membrane level (Kras probe) (Cf. figure 1): following the exposure to the agonist dopamine, we observe a significant decrease of BRET signal compared to the basal (untreated cells), and this signal is totally reversed by the antagonist haloperidol. Aripiprazole promotes only a partial response (a). The stimulations by dopamine and aripiprazole are concentration-dependent and present similar potencies (b).

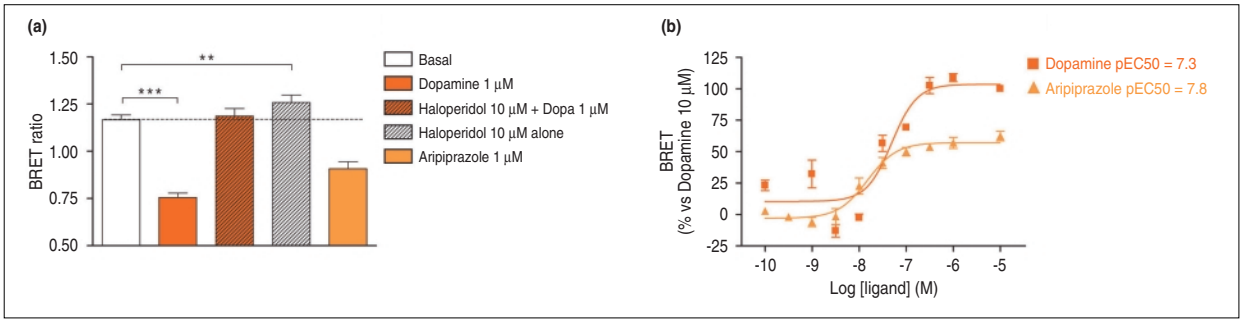

Figure 1: Measurement of energy transfer (BRET) between D2s receptor and Kras in living cells.
BRET is measured in CHO cells coexpressing D2s-Rluc8 and GFP²-Kras. Data represent the mean ± s.e.m. of at least 3 independent experiments. Statistics were performed using a "paired Student t-test"

Conclusions

By positioning a light-emitting probe at the plasma membrane (Kras), we have implemented a new tool based on BRET^2 technology allowing us to monitor in real-time and in living cells the internalization of the D2s receptor after its stimulation. This work confirms the ability of FDSS/μCELL to monitor protein-protein distance changes using BRET^2 technology.

Bibliography

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