

GALANIN RECEPTOR 2 MODIFIES NEUROPEPTIDE Y Y1 RECEPTOR INTERNALIZATION AND β -ARRESTIN RECRUITMENT

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We have recently described a Galanin receptor 2(GALR2) and Neuropeptide Y Y1 receptor(NPYY1R) interaction at behavioural, cellular and receptor levels through GALR2/NPYY1R heterodimers. The aim of this work was to study if GALR2 and NPYY1R costimulation modified NPYY1R internalization and β -Arrestin recruitment after in HEK293T cells.

HEK293T cells were transfected with NPYY1R^{EGFP} or β -Arrestin2^{GFP2} cloned with standard molecular biology techniques employing PCR and fragment replacement strategies. NPYY1R^{EGFP}/GALR2 and NPYY1R/GALR2 with β -Arrestin2^{GFP2} HEK293T coexpressing cells were incubated with NPY 1 μ M and/or GAL1 μ M, at different times. Antagonist studies were performed 15 min prior to the addition of agonist with NPYY1R antagonist BIBP3226 10 μ M or GALR2 antagonist M871 10 μ M. Timed-interval images of NPYY1R^{EGFP} or β -Arrestin2^{GFP2} endosomes in different cell groups were acquired using a confocal microscope following agonist addition. Percentage of internalization was determined by Leica software analysis of total membrane fluorescence compared to total internal compartment fluorescence at the various time points.

We observed that addition of NPY induced a rapid decrease in the cell surface expression of NPYY1R^{EGFP} and a redistribution of β -Arrestin2^{GFP2}. In fact, we observed a maximum of internalization of 80% three minutes after the NPY stimulation. However, combined treatment with GAL and NPY induced a delay in the internalization of NPYY1R^{EGFP}, with a maximum of internalization thirty minutes after the co-stimulation. Moreover, a delay in the β -Arrestin2^{GFP2} redistribution was observed. The specific GALR2 antagonist M871 abolished these delays in internalization of NPYY1R^{EGFP} and β -Arrestin2^{GFP2} redistribution, suggesting that this effect was mediated through the coactivation of GALR2 and NPYY1R. These results demonstrate that costimulation with GAL and NPY delays the internalization of

NPYY1R^{EGFP} by decreasing recruitment of β -Arrestin2^{GFP2} and probably could change intracellular signaling. This study was supported by Junta de Andalucía CVI6476.

Áreas Temáticas:

1ª: Neurociencia cognitiva y conductual