

Galanin interacts with Neuropeptide Y Y1 receptor in the dentate gyrus of the hippocampus through GALR2/NPYY1R heterodimers

We have shown Galanin(GAL) and Neuropeptide Y Y1(NPYY1) interactions at behavioural, cellular and receptor levels through GALR2/NPYY1R heterodimers in the amygdala. The aim of this work was to analyze GAL/NPYY1R interactions in the Dentate Gyrus(DG) of the Hippocampus, using autoradiographic, *in situ* hybridization and *in situ* proximity ligation assay(PLA).

Rats(n=6) were sacrificed 15 minutes or 5 hours after icv injections of GAL(3nmol) and DG sections were incubated with NPYY1R agonist [¹²⁵I]-[Leu³¹,Pro³⁴]PYY(25 pM) or NPYY1R-³³PdATP specific probe, for autoradiography and *in situ* hybridization respectively. Autoradiograms were analyzed using NIH image analysis system and Student's unpaired t-test was used. For PLA, DG sections were incubated with anti-GALR2 Rabbit(1:100) and anti-NPYY1R Goat(1:200). PLA signals were detected with PLA PLUS or MINUS probes for rabbit or goat/mouse antibodies. PLA signals were visualized by using a confocal microscope Leica TCS-SL confocal microscope(Leica).

We observed that GAL significant increased the NPYY1R agonist [¹²⁵I]-[Leu³¹,Pro³⁴]PYY binding in the DG by 20% (p<0,05) and the NPYY1R mRNA expression in the granular layer of DG by 31% (p<0,001). Moreover, PLA-positive red clusters were found specifically in the polymorphic layer and subgranular zone of the DG. No PLA clusters were observed neither in the molecular layer of the DG nor in the corpus callosum, an area that seems to lack of GALR2 receptor.

These results demonstrate a novel mechanism of interaction between GAL and NPY1R in the DG at receptor level, probably involving the formation of GALR2/NPYY1R heteroreceptor complexes. Study supported by Junta de Andalucía CVI6476.