

ANTIDEPRESSANT-LIKE EFFECTS INDUCED BY GALANIN 2 /NEUROPEPTIDE Y Y1 HETERODIMERS IN THE DENTATE GYRUS OF THE HIPPOCAMPUS

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Previously, we have described the Galanin (GAL) and Neuropeptide Y Y1 (NPYY1) interactions through GAL receptor 2 and NPYY1 receptor 1 (GALR2/NPYY1R) heterodimers in the Dentate Gyrus (DG) of the Hippocampus, using autoradiographic, *in situ* hybridization and *in situ* proximity ligation assay (PLA) (1,2). The current work is to evaluate GALR2 and NPYY1R interactions in relation to depression-like behaviour and c-Fos expression in the Hippocampal DG.

Rats (n=6-8) were forced to swim for a 15-min period (pre-test) and 24 h later were subjected to a 5-min swimming session (test) 15 min after the administration alone or in combination of GAL, the NPYY1R agonist [Leu³¹,Pro³⁴]NPY and the GALR2 antagonist M871. The total duration of immobility, swimming, and climbing periods were scored during the test. For c-Fos immunohistochemistry, experimental groups of rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused with 4 % Paraformaldehyde 90 min after icv injections. Then, brains were coronally sliced and immunostained. As primary antibodies, an antibody against c-Fos protein (1:5000, sc- 52, Santa Cruz Biotechnology, CA, USA), revealed with 3,3'-Diaminobenzidine (DAB) plus nickel, was used as an indirect marker of neural activity. The antibody to Calbindin-D28 k (1:1000, Santa Cruz Biotechnology, CA, USA), revealed with DAB, was used to outline the granular region since it marks mainly hippocampal granule cells. Sections were analyzed using the optical fractionator stereological method.

We observed that icv injection of GAL and NPYY1R agonist significantly enhanced the decrease in the immobility ($p < 0,001$) and the increase in the swimming behaviour ($p < 0,001$) compared with the NPYY1R agonist alone. Moreover, a significant enhancement of the decrease in climbing behavior ($p < 0,05$) was also observed. Furthermore, GALR2 is involved in this GALR/NPYY1R interaction, since the presence of the GALR2 antagonist M871 counteracted the enhancement of the decrease in immobility ($p < 0,01$) and in climbing behavior ($p < 0,05$) as well as the

increase in swimming time ($p < 0.001$) induced by the coadministration of GAL and NPY1R agonist in the FST.

Specific cells populations within DG subregions may be involved in this behavioural effect since the coadministration of GAL and NPY1R agonist enhances the NPY1R-mediated reduction ($p < 0.05$) in the number of c-Fos immunoreactive nuclei in the polymorphic region. In this region, the GABA interneurons could be involved in the interaction since c-Fos IR colocalized with a GABAergic marker (GAD65/67) after NPY1R agonist injection. Moreover, within the granular cells layer, GAL and NPY1R agonist coadministration significantly increased c-Fos IR expression in the entire granular cell layer compared with GAL ($p < 0.05$) and [Leu³¹,Pro³⁴]NPY ($p < 0.01$) alone. Again, the co-treatment with the GALR2 antagonist M871 completely reversed the GAL contribution to the responses in both regions, the polymorphic and the granular layer of the DG, demonstrating the involvement of GALR2 in the GAL actions.

These results indicate that GALR2/NPY1R interactions can provide a novel integrative mechanism in DG in depression-related behavior and may give the basis for the development of drugs targeting GALR2/NPY1R heteroreceptor complexes in the DG of the hippocampus for the treatment of depression. Study supported by Junta de Andalucía CVI6476 and Proyecto Puente-Universidad de Málaga.

1. Galanin receptor 2-neuropeptide Y Y1 receptor interactions in the dentate gyrus are related with antidepressant-like effects. Narváez M, Borroto-Escuela DO, Millón C, Gago B, Flores-Burgess A, Santín L, Fuxe K, Narváez JA, Díaz-Cabiale Z. *Brain Struct Funct* 2016 Nov. 221(8):4129-4139.

2. Galanin receptor 2-neuropeptide Y Y1 receptor interactions in the amygdala lead to increased anxiolytic actions. Narváez M, Millón C, Borroto-Escuela D, Flores-Burgess A, Santín L, Parrado C, Gago B, Puigcerver A, Fuxe K, Narváez JA, Díaz-Cabiale Z. *Brain Struct Funct*. 2015 Jul;220(4):2289-301.