Free fatty acid receptor 4 activation induces lysophosphatidic acid receptor 1 (LPA1) desensitization independent of LPA1 internalization and heterodimerization

A. Meizoso-Huesca,[1,2] S. Villegas-Comonfort,[1] T. Romero-Avila[1] and A. Garcia-Sainz,[1] [1]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, C.U., Av. Universidad Nº 3000, 04510 México City, México and [2]School of Biomedical Sciences, The University of Queensland, St, Lucia, QLD 4072, Australia.

Lysophosphatidic acid is one of the main mitogenic compounds of blood serum. Its functions are mediated by a family of 6 GPCRs (known as LPA_{1-6}) expressed differentially among the tissues of the body. On the other hand, the Free Fatty Acids Receptor 4 (FFA4) is a well-known GPCR of long chain free fatty acids that has been related to anti-diabetic and anti-inflammatory processes as well as brain development.

In different cancer types (prostate, breast, and ovary), FFA4 activation has been proven to block mitogenic features of LPA_1 activity. In consequence, the crosstalk between these receptors is of pathophysiological relevance.

The aim of this study was to explore the crosstalk between LPA and FFA4 employing co-expression of fluorescent protein-tagged receptors on HEK 293 cells. Functional FFA4-mediated LPA_1 desensitization was assessed by calcium fluorometry of cells in suspension and western blotting. As a classic negative-regulation modification, phosphorylation of receptors in response to their agonists was tested: FFA4 activation induced phosphorylation of both receptors. As expected, LPA_1 activation induced phosphorylation of LPA_1, but not of FFA4. LPA_1 activation drove internalization of both receptors into heterogeneous types of vesicles. FFA4 agonist led to internalization of FFA4 but not of LPA_1, suggesting a desensitization mechanism independent of the internalization of the receptor. Dimerization of GPCRs in response to a stimulus has been shown to induce modifications in pharmacological properties of these proteins, leading to changes of the affinity for their ligands. In order to test this, fatty acid-induced FFA4-LPA_1 interaction was observed using Forster Resonance Energy Transfer (FRET) and co-immunoprecipitation; however, such interaction took place once the desensitization was already established. Data indicate that FFA4 activation induces LPA_1 desensitization in an internalization-independent mechanism and that during the first moments of this desensitization, heterodimerization does not play a relevant role.