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Analysis of interaction between dimerized receptor and β-arrestin

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[Background] G-protein coupled receptors (GPCR) are known to form a dimerized homomeric or heteromeric receptors. However, coupling between dimerized receptor and β-arrestin is not well understood. We previously found that vasopressin can modulate morphine tolerance in ventral medulla through V1b receptor. However, how V1b, μ opioid receptors and β-arrestin 2 are arranged is not known. Here, we employed three molecule BRET (bioluminescence resonance energy transfer), which occur between split luciferase and green fluorescent protein (GFP), to monitor association of a receptor-containing complex. [Method] V1b and μ opioid receptors were connected at their carboxyl-termini to each part of split luciferase. β-arrestin 2 was connected to an enhanced green fluorescent protein. HEK cell were transfected with genes for receptors and β-arrestin 2. After agonist stimulation, BRET signal was measured by a plate reader. [Results] Receptors connected with split luciferase were functional in term of generating their cellular responses. V1b and μ opioid receptors formed homomeric or heteromeric receptor dimers, which were detect through luciferase intensities. Significant BRET signal was generated by interaction between receptor dimer and β-arrestin 2. [Conclusions] Our data suggested that the three-molecule BRET analysis can be applied to study interaction between receptor dimer and β-arrestin.

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