

P001 Interaction between the β_2 -adrenergic receptor and arrestin is dependent on both receptor phosphorylation and the presence of agonists.

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Many G-protein-coupled receptors are phosphorylated upon agonist stimulation by G-protein-coupled receptor kinases (GRKs) and subsequently bind arrestins, a process that leads to receptor desensitization. We have investigated the interactions between β_2 -adrenergic receptors (β_2 ARs) and arrestin3 in single living cells. Using confocal microscopy, we confirm previous findings that interaction of arrestin3 with the β_2 AR requires serine and/or threonine residues between amino acids 355 and 364 but is independent from serine and threonine residues at the extreme C-terminus. By measuring FRET between YFP-tagged β_2 ARs and CFP-tagged arrestin3 we show that the initial kinetics of arrestin3 binding to the β_2 AR is limited by the kinetics of GRK2-mediated receptor phosphorylation. Agonist withdrawal causes immediate dissociation of the receptor-arrestin complex. Subsequent stimulation of the same cell leads to much faster arrestin3 binding to the receptor. We show that this rapid association is probably caused by the existence of pre-phosphorylated receptors at the plasma membrane. Our findings suggest a fast agonist-controlled interaction between arrestins and pre-phosphorylated receptors which may permit rapid control of receptor sensitivity in repeatedly stimulated cells such as neurons.