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Mass spectrometry identification of phosphorylation sites in the PTH1R reveals residues responsible for high affinity interaction with arrestin-3 and fine tuning downstream signaling

Phosphorylation of G-protein coupled receptors constitutes a key event in regulating receptor function and determines receptor desensitization as well as arrestin recruitment. It is supposed that different phosphorylated single residues or entire phosphorylated clusters not only induce distinct extents of arrestin recruitment but also elicit specific arrestin conformations. In the present study using mass spectrometry and mutagenesis we identified ten residues of parathyroid hormone receptor 1 (PTH1R) undergoing phosphorylation (S73, S473, S491, S492, S493, T503, S504, S519, T547, and T551), three of which have not been described so far (pT503, pS519 and pT547). Mutational analysis of these residues revealed that 50% of total ligand-induced phosphorylation was mediated by the first large serine cluster (S489-S495) in the proximal part of the c-terminal tail. Using FRET and BRET we show that the first cluster S489-S495 and the second cluster S501 T506 operated in concert to mediate both the efficacy and potency of ligand-induced arrestin-3 recruitment. Interestingly, further analysis including FRAP demonstrated that intact phosphorylation of S503 and T504 in the second cluster contributed to 60-70% of arrestin-3 recruitment and was decisive for the high affinity interaction of arrestin with the receptor. Furthermore, measuring cAMP we found that both the wild type receptor and a mutant deficient in phosphorylation (S489-S495) were similar sensitive for PTH 1-34, the mutant however, displayed a higher constitutive activity in comparison to the wild type receptor. Investigating the impact of PTH1R phosphorylation on downstream signaling we found that subsequent removal of serine and threonine clusters in the receptor c-terminus increased and prolonged the activation of the ERK1/2 pathway. In summary, this study identified three phosphorylation sites in the PTHR which have not been described so far. Furthermore we demonstrate the significance of PTH1 receptor phosphorylation patterns in regulating PTH 1-34 mediated arrestin affinity and fine tuning ERK1/2 activation.