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Mutation of lysines to arginines in the arrestin N-terminus lowers arrestin affinity to GPCRs by attenuating the release of the arrestin C-terminus

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Introduction The mutation K11,12R (KKRR) in arrestin3 has been reported to reduce intracellular trafficking of arrestin with some but not all G protein-coupled receptors (GPCRs). This has been attributed to reduced ubiquitination of arrestin3 KKRR (1). However, molecular modelling suggested that Arg but not Lys residues in these positions can interact with the C-terminus of arrestin, thereby preventing or slowing the release of the arrestin C-terminus necessary for full activation. We investigated whether we could convert the binding of the arrestin KKRR mutant back to wild-type by facilitating the release of the C-terminus.

Methods Using the Quickchange protocol, we introduced additional mutations Glu389 and Asp390 to Ala in the Arr3 KKRR mutant (KKRR EDAA) to disrupt the interaction of R11,12 with the C-terminus. The affinity of the arrestin mutants to different GPCRs, the β2 adrenoreceptor SSS (2), the chimeric β2V2 receptor and the parathyroid hormone receptor 1 (PTHR), was analyzed in FRET (Förster Resonance Energy Transfer) and dual-colour FRAP (Fluorescence Recovery After Photobleaching) measurements. Furthermore, co-internalization of receptors and arrestins was visualized by confocal imaging. All experiments were performed as published before using YFP-labelled receptors and CFP-labelled arrestins expressed in HEK293T cells (2).

Results FRAP experiments showed that Arr3 KKRR displayed significantly lower affinity to the β2V2 receptor (n=33, k-values WT 0.117 ± 0.008, KKRR 0.071 ± 0.009 s^{-1}, $p<0.05$, student's t-test). However, the additional mutation ED389AA could rescue the affinity of the KKRR mutant (n=33, k-value 0.121 ± 0.021 s^{-1} $p<0.05$, student's t-test vs KKRR). FRET measurements confirmed that this mutation could convert the arrestin affinity back to wild-type. Analyzing the co-internalisation of arrestin and β2AR-SSS (2) with confocal microscopy we found that Arr3 KKRR, in contrast to wild-type, did not internalize in endosomes after receptor activation (n=15, Pearson coefficient receptor-arrestin WT 0.478 ± 0.022, KKRR 0.128 ± 0.014, $p<0.0001$, student's t-test). Again, this loss of affinity was not observed with Arr3 KKRR EDAA mutant, as it co-internalized with the receptors (n=15, Pearson coefficient receptor-arrestin 0.461 ± 0.019, $p<0.0001$, student's t-test vs KKRR).

Conclusion We conclude that the K11,12R mutant does not have a lower affinity to GPCRs because the Arg residues cannot be ubiquitinated but rather because the release of the arrestin C-terminus and arrestin activation are attenuated by intramolecular interactions.

References

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(2) Zindel D *et al.* (2015). *Mol Pharmacol* **87**: 349-362