**GRK2 inhibits the interaction of the $\alpha_{2A}$-adrenoceptor with $G_i$ in a phosphorylation-independent manner**

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*Introduction*: Most G-protein-coupled receptors (GPCRs) undergo homologous desensitisation after agonist stimulation. This two-step process involves phosphorylation of the agonist-occupied receptor by G-protein-coupled receptor kinases (GRKs), followed by binding of arrestins to the still agonist-occupied, phosphorylated receptor. We wanted to study homologous desensitisation of the $\alpha_{2A}$-adrenergic receptor ($\alpha_{2A}$AR) by investigating the effects of GRKs and arrestins on G-protein recruitment.

*Methods*: We measured FRET between YFP-tagged $\alpha_{2A}$AR and CFP-tagged Gβ in transiently transfected HEK293T cells which were stimulated twice with 10 µM norepinephrine, and evaluated the association kinetics.

*Results*: We expected that cotransfection of GRKs and arrestins would have little effect on the initial interaction kinetics of the $\alpha_{2A}$AR with $G_i$ but would slow it down during the second stimulation. However, we found that cotransfection of GRK2 and arrestin3 already slowed down the initial interaction kinetics; the half-life of the receptor:G-protein interaction was 0.2 s (n=10) in the absence of GRK2/arrestin3 but 2.3 s (n=12) in the presence of GRK2+arrestin3 (one-way ANOVA with Dunn post-hoc test: $p < 0.001$). This effect was independent of arrestin3 (half-life of 1.8 s, n=7, $p>0.05$). No arrestin-independent effect of GRK2 only was observed with the $G_s$-coupled $\beta_1$-adrenergic receptor. The effect could also not be recapitulated using GRK5 or GRK6 which showed half-lifes of 0.4 s (n=9 for GRK5, n=13 for GRK6). In the presence of the GRK2 C-terminus, well known to bind Gβγ subunits, the half-life of Gβ recruitment to the $\alpha_{2A}$AR was slightly longer (0.6 s, n=13) but still significantly faster than in the presence of full-length GRK2 ($p < 0.05$). Interestingly, GRK2 catalytic activity was not required for its effect on Gβ recruitment to the $\alpha_{2A}$AR as there was no significant difference between GRK2 and GRK2 K220R ($p > 0.05$).

*Conclusions*: GRK2 can inhibit the interaction between the $\alpha_{2A}$AR and $G_i$ in a phosphorylation-independent manner. We are currently investigating whether this effect can be observed for other $G_i$-coupled GPCRs and which regions in GRK2 mediate the inhibition.