Gi protein activity is sensitised by type 5 adenylyl cyclase (AC5)

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The second messenger cAMP is synthesised by adenylyl cyclases (AC). ACs themselves are regulated through receptors and G proteins. Here we investigated the dynamic interactions between the alpha-subunit of the type 1 inhibitory G protein ($G\alpha_{i1}$) and human type 5 adenylyl cyclase (AC5). To allow detection, these proteins were labelled with CFP and YFP, respectively. YFP was fused to the N-terminus of AC5 using the 3-fragment Multisite Gateway Pro cloning system by Invitrogen. YFP was cloned into $G\alpha_{i1}$ between amino acids 91 and 92 as described previously (Buenemann et al., PNAS 2003). CFP-$G\alpha_{i1}$ was constructed accordingly. The interaction dynamics were recorded with high temporal resolution by means of FRET in HEK cells. The cells additionally expressed unlabelled α_{2A} adrenoceptor and Gβγ subunits to allow the studying of agonist induced changes in the interaction. During the experiments the cells were superfused with either agonist/buffer-solutions in different concentrations or agonist-free buffer to wash out the agonist between applications and to allow the interaction to recover to baseline. Upon application of noradrenalin a developing FRET signal could be observed, which indicates the interaction between $G\alpha_{i1}$-CFP and YFP-AC5. In contrast to previous results on the interaction kinetics of Gi proteins the interaction between $G\alpha_{i1}$-CFP and YFP-AC5 recovered with a remarkably slow time course after the agonist was withdrawn. For further investigation we compared the interaction between $G\alpha_{i1}$ and AC5 with the interaction between $G\alpha_{i1}$-YFP and Gβγ-CFP (Gi-FRET). Furthermore we tested the acceleration of deactivation of Gi proteins and the dissociation of $G\alpha_{i1}$-CFP from YFP-AC5 by overexpression of RGS4. The dissociation of $G\alpha_{i1}$-CFP from YFP-AC5 after agonist withdrawal takes about 2 times longer than the deactivation of the Gi protein itself (half-life of 55.1±4.3 s and 29.3±3.7 s, respectively). While RGS4 accelerates the Gi protein deactivation it has hardly any effect on the dissociation of $G\alpha_{i1}$-CFP from YFP-AC5 (half-life 15.8±1.7 s and 48.3±4.3 s, respectively). From this we hypothesised that AC5 possibly traps active G proteins which would affect the equilibrium in the G protein cycle and shift it towards a higher amount of active G proteins. Should this hypothesis be correct, the concentration-response curve of the interaction between $G\alpha_{i1}$-CFP and YFP-AC5 should be shifted leftwards compared to the concentration-response of the G protein activation. Indeed, we found the concentration-response curve for the $G\alpha_{i1}$-AC5 interaction to be shifted leftwards in comparison to the G protein activation concentration-response ($G\alpha_{i1}$-AC5 interaction: $\log(EC_{50})$=-8.52±0.13 log(M); Gi protein activation: $\log(EC_{50})$=-8.05±0.22 log(M)). Effector-specific influence on the dynamics of the G protein cycle may represent a powerful mechanism to fine-tune receptor evoked responses. (Results are presented as mean with SEM of at least 10 individual experiments.)