7 SUMMARY

The stimulatory guanine nucleotide-binding protein (G_S) transmits signals from stimulatory G protein-coupled receptors (GPCR) to adenylyl cyclases, thereby increasing the production of the second messenger cAMP. Four splice variants of the α-subunit of G_S ($G\alpha_S$) are expressed in mammalian tissues. Numerous studies have analysed their function, but due to differing experimental parameters and systems, the results were neither consistent nor comparable. Differences in the biochemical properties of heterologously expressed, purified splice variants have been described, but it is not known whether they are enhanced or weakened by transmission through the full signalling cascade. Therefore, the effectiveness of signal transduction via the four splice variants was compared using specific repression (knock down) or reconstitution of unmodified proteins in $G\alpha_S$-deficient cell lines.

Several methods of transient repression were compared using a luciferase reporter system. Catalytically active DNA (DNAzyme) was unsuitable because of its low activity at physiological magnesium concentrations. In contrast, up to 90 % inhibition of luciferase expression was achieved by either propyne-modified oligodeoxynucleotides or intracellular expression of small interfering RNA. The propyne-modified oligodeoxynucleotides were chosen for inhibition of $G\alpha_S$ on account of their greater flexibility in the choice of target sequences. They inhibited expression of transiently transfected but not of endogenous $G\alpha_S$.

In Sf9 insect cells, $G\alpha_S$ splice variants were co-expressed with various GPCR. The interaction between GPCR and $G\alpha_S$ in plasma membranes was compared by measuring ligand-dependent binding kinetics of the GTP analogue [^{35}S]GTPγS. The four splice variants were activated with comparable apparent rate constants by the β_2-adrenergic, glucagon, histamine and secretin receptors ($k_{app} \approx 0{,}1$ min^{-1}).

In the $G\alpha_S$-deficient mouse fibroblast cell line 2B2, the signal transduction cascade GPCR – $G\alpha_S$ – adenylyl cyclase was reconstituted by transiently expressing $G\alpha_S$ splice variants. Adenylyl cyclase activity was measured in the plasma membranes. Direct activation of $G\alpha_S$ splice variants showed them to be comparably effective and potent in activating adenylyl cyclase with half-maximal effective concentrations of 0.24-0.31 nM. Due to the similarity of these values, adenylyl cyclase activity could be taken as a measure of the interaction between GPCR and $G\alpha_S$. The splice variants were comparably potent in

receptor-mediated adenylyl cyclase activation by the β_2-adrenergic, glucagon, histamine, secretin, vasopressin, and luteinising hormone receptors. However, the reason for the expression of four different splice variants remains to be elucidated; interactions with other signalling molecules such as tubulin and Src family tyrosine kinases are discussed.