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Mutations of Cys285$^{6.47}$ of the β_2 Adrenergic Receptor Modulate the PRO-KINK in TM6 and Produce Constitutive Activity

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AIM

Biophysical and biochemical studies have shown that activation of G-protein-coupled receptors is associated with a movement of transmembrane segment (TM) 6 relative to TM3. Recently, we proposed that this conformational change involves an alteration in the extent of the Pro-kink in TM6, with the more bent state representing the inactive state. In the present work we hypothesized that in the β_2 adrenergic receptor (β_2AR) the highly conserved Cys285$^{6.47}$, i-3 from Pro288$^{6.50}$, might modulate the Pro-kink, and that mutation of this residue might alter the constitutive activity of the receptor.

RESULTS

Binding studies and cAMP accumulation studies showed that the C285$^{6.47}$T mutation constitutively activated the β_2AR, thus resulting in a) an increase of isoproterenol affinity, 5 fold, b) an increase of isoproterenol potency, 4 fold and c) a significant increase of receptor mediated basal cAMP accumulation, which was blocked by the inverse agonist timolol. In contrast, C285$^{6.47}$S and C285$^{6.47}$A mutations did not significantly alter binding or activation properties.

CONCLUSION

In conclusion, we suggest that a straightening of the Pro-kink in TM6 induced by C285$^{6.47}$T swings TM6 away TM3, thereby disrupting interactions at the cytoplasmic ends of TM3 and TM6 and producing constitutive activity. In order to probe the changes in conformational space resulting from these mutations, we are implementing Monte Carlo simulations with conformational memories and an implicit solvent model on polypeptides mimicking TM6 of β_2AR and the mutants.