Cloning of chimaeric orexin receptors

Orexins, orexin-A and -B, are recently found peptide transmitters/hormones. They regulate physiological processes via two G-protein-coupled receptors called OX_1 and OX_2 receptors. Orexins are very central for the regulation of wakefulness and REM sleep and feeding, and the collapse of the orexinergic system results in the sleep disorder narcolepsy.

Orexin receptors are thus of high interest what concerns basic knowledge of the central physiological processes of sleep, feeding and metabolism and also development of therapeutic strategies for disorders of these. One central tool would be selective compounds, both agonist and antagonists, for each receptor subtype. We have previously performed mutagenesis of orexin-A in order to determine the requirements made by each receptor subtype (Ammoun et al., J. Pharmacol. Exp. Ther., 2003). Unfortunately, the study was not very conclusive, i.e. the same mutations in orexin-A peptide affected binding and activation of both receptor subtypes. We therefore designed an approach to mutate the orexin receptors instead. The aim of this study is to create chimaeric orexin receptors, using PCR technique, composed of the front part of the OX_1R and the rear of the OX_2R and *vice versa*. The idea is that the chimaeric receptors should show where the binding of the orexin peptide, orexin-A and orexin-B, resides in each of the receptors. Orexin receptors, while being non-selective for orexin-A, show selectivity with respect to orexin-B, and thus the results of this study can assist in the development of receptor subtype selective ligands. The GFP-tagged chimaeras will be expressed transiently and the functionality will be assed using Ca^{2+} and inositol phosphate release measurements.

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