REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 24: 144 (2010)

Probing the structure of the extracellular portion of the third membrane-spanning segment of CRF_1 using the substituted-cysteine-accessibility method.

Kostas Gkountelias, Mary Papadokostaki and George Liapakis

Department of Pharmacology, Faculty of Medicine, University of Crete, Heraklion, Crete, GR

Key words: Corticotropin-releasing factor type 1 receptor, structure, third membrane-spanning segment, binding-site crevice, methanethiosulfonate ethylammonium

SUMMARY

The type 1 receptor (CRF_1) for the corticotropin releasing factor (CRF) belongs to family B of G-protein coupled receptors (GPCRs) and, like the other GPCRs, consists of seven membrane-spanning segments (TMs), which have been proposed to bind small non-peptide ligands. Recently we have shown that, similar to family A, GPCRs, the TMs of CRF_1 form a water-accessible crevice, the binding-site crevice, which extends from the extracellular surface of the receptor into the plane of the membrane. The surface of this crevice must be formed by residues that contact ligands, as well as, by other residues that may play a structural role and affect binding indirectly. In this study we mapped the TM residues that form the surface of the binding-site crevice of CRF_1, starting from the extracellular portion of the third TM (TM3). We achieved this by applying the cysteine-substituted accessibility method (SCAM) and using as background the ΔCys mutant of CRF_1, which has near normal functional properties and it is relatively insensitive to the methanethiosulfonate (MTS) reagents. We mutated eight TM3 residues of CRF_1 to Cys and heterologously expressed the mutants in HEK 293 cells. Four of these mutants reacted with the hydrophilic, positively charged sulfhydryl-specific reagent, methane-thiosulfonate ethylammonium (MTSEA), added extracellularly. We therefore suggest that the side chains of the residues at the reactive loci (Thr192, Ala193, Tyr195, and Asn196) are on the water-accessible surface of the binding-site crevice of CRF_1. The pattern of accessibility is consistent with an alpha-helical conformation for this portion of TM3.