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Residues at Positions 211, 233 and 364 of Crf_1 Are Exposed in the Binding-Site Crevice of Receptor

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SUMMARY

The type 1 receptor (CRF_1) for the corticotropin-releasing factor (CRF) belongs to family B of G-protein-coupled receptors (GPCRs). The CRF_1, like all GPCRs, is a protein that spans the plasma membrane seven times thus forming seven membrane-spanning segments (TMs), which have been proposed to bind small non-peptide ligands, such as antalarmin. This leads to the hypothesis that similar to family A, rhodopsin-like, GPCRs, the membrane-spanning segments of CRF_1 as well as all family B GPCRs 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 is formed not only by residues that can contact small ligands but also by residues that may play a structural role and affect binding indirectly. However, the lack of considerable structural information for the family B GPCRs precludes the support of this hypothesis. To test this hypothesis we started obtaining information about the structure of family B GPCRs, using as prototype the CRF_1 and testing its reaction with the positively charged sulfhydryl-specific methanethiosulfonate ethylammonium (MTSEA). We found that MTSEA inhibited the binding of the radiolabelled CRF analog, [^{125}I]-Tyr^0-sauvagine, to CRF_1, and that antalarmin protected against this irreversible inhibition. To identify the susceptible cysteine(s), we mutated, one at a time, four endogenous cysteines to serine. Mutation of Cys211, Cys233, and Cys364 to serine decreased the susceptibility of sauvagine binding to irreversible inhibition by the MTSEA. Thus, Cys211, Cys233 and Cys364 at the cytoplasmic ends of the third, fourth and seventh membrane-spanning segments are exposed in the binding-site crevice of CRF_1. These studies will ultimately provide us with the required information for the structure of CRF_1 and will be used to construct a CRF_1 molecular model. This model will be used as a prototype, for the understanding of the structure and function of all receptors belonging to the family B of GPCRs. This molecular model will also help us to determine the residues in the membrane-spanning segments of CRF_1 that interact with small non-peptide ligands, thus putting the basis for the rational design of new small CRF_1-selective ligands.