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A new approach for studying GPCR dimers: Drug-induced inactivation and reactivation to reveal GPCR dimer function in vitro, in primary culture, and in vivo

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ABSTRACT

GPCRs are a major family of homologous proteins and are key mediators of the effects of numerous endogenous neurotransmitters, hormones, cytokines, therapeutic drugs, and drugs-of-abuse. Despite the enormous amount of research on the pharmacological and biochemical properties of GPCRs, the question as to whether they exist as monomers, dimers, or higher order structures in the body is unanswered. The GPCR dimer field has been dominated by techniques involving recombinant cell lines expressing mutant receptors, often involving the solubilization of the receptors. These techniques cannot be applied in vivo or even to primary cell cultures. This review will focus on a novel approach to exploring the functional properties of homodimers. Studies of the 5-HT_7 and 5-HT_{2A} serotonin receptors have revealed that binding of a pseudo-irreversible antagonist ("inactivator") to one of the orthosteric sites of a homodimer abolishes all receptor activity, and subsequent binding of a competitive antagonist to the orthosteric site of the second protomer releases the inactivator, allowing the receptor to return to an active state. This approach demonstrates allosteric crosstalk between protomers of native GPCR homodimers, indicating that GPCRs do exist and function as homodimers in both recombinant cells and rat primary astrocytes. This technique can be applied universally using intact recombinant or primary cells in culture, membrane homogenate preparations and, potentially, in vivo. The data obtained using the 5-HT_7 and 5-HT_{2A} receptors are strongly supportive of a GPCR homodimer structure, with little evidence of monomer involvement in the function of these receptors.

Contents

1. Introduction

1.1. The importance of studying G-protein coupled receptor dimers

There is a growing sense that G-protein coupled receptors (GPCRs) may be obligatory dimers in vivo (Fig. 1). Thus, the commonly depicted seven transmembrane (7TM) structure for a GPCR actually represents a subunit of the overall macromolecular structure.

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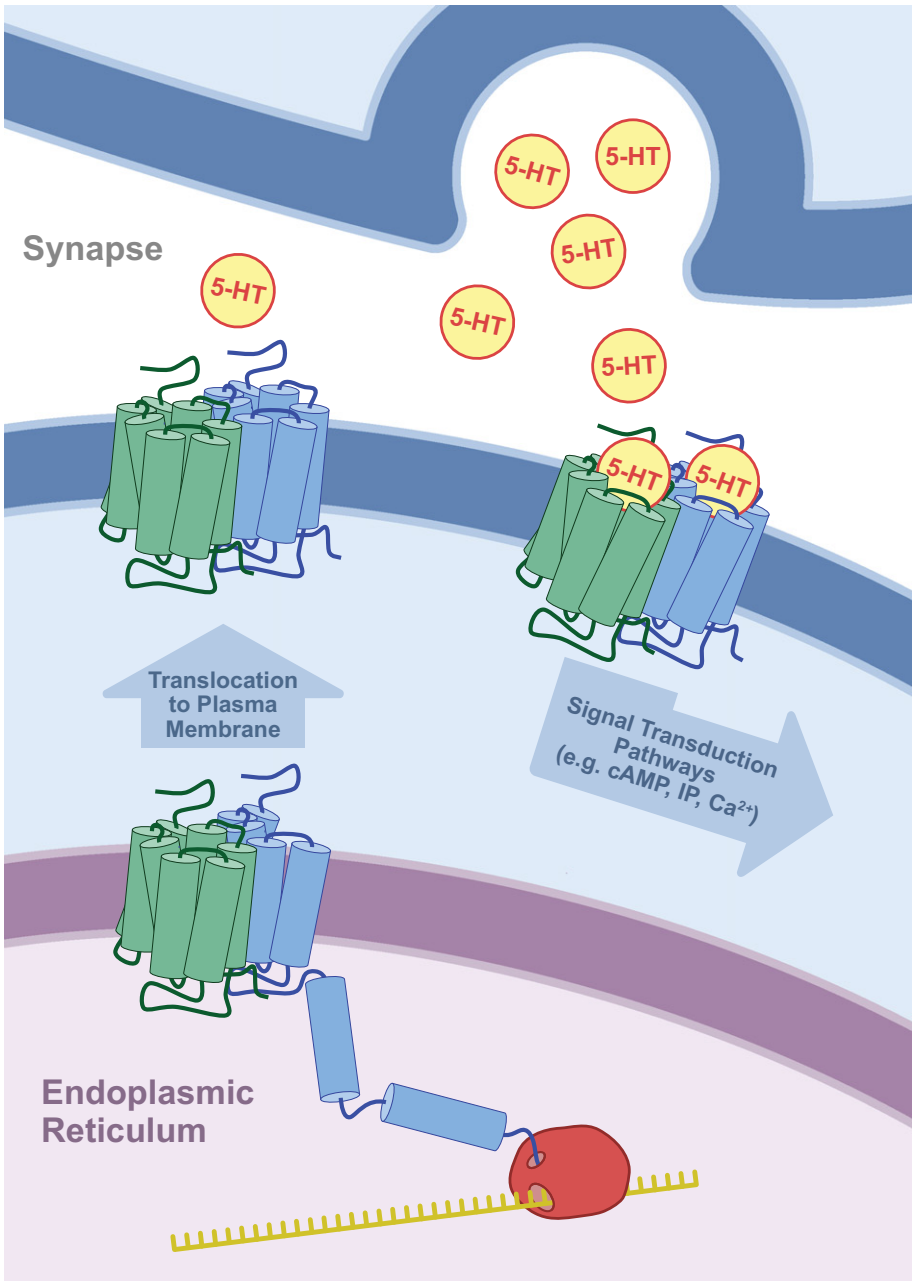

Fig. 1. The obligatory dimer GPCR model. In order for the GPCR to be expressed in a functional state on the plasma membrane, an intracellular dimer must be formed. The dimer may be a homodimer or a heterodimer. Thus, a GPCR may be viewed as containing two "subunits", each a distinct gene product. This illustration depicts neuronal serotonin (5-HT) receptors, but may be applied to any GPCR.

Developing a simple, definitive, relatively non-invasive methodology for detecting homodimer or heterodimer-associated functional effects of native GPCRs in vivo is essential if the question of the prevalence and function of GPCR oligomers is to be answered. Besides the importance, from a basic cell biology viewpoint, of knowing the quaternary structure and function of GPCRs, there are important clinical implications. The processing and positioning of GPCRs have been implicated in disease processes, and strategies for altering these processes have been targeted for novel therapeutic drug development (Rene et al., 2010). Therefore, the processing and positioning of a GPCR oligomer will involve numerous cell biological functions not yet investigated in vivo. Dysfunction in the processing and/or the functioning between the protomers of a GPCR dimer or higher order structure could be responsible for disease states (Wang et al., 2010). As we have no reliable methodologies to investigate GPCR oligomer function in vivo, this is a wide-open area of investigation. Drugs that change the processing and/or function of the GPCR oligomer may prove to be novel therapeutics.

1.2. Overview of the G-protein coupled receptor dimer field: the recombinant cell line "bottleneck"

The exploration of the molecular properties of the GPCR family of receptors began in the 1970s and resulted in a universally accepted receptor/GTP-binding-protein/adenylate cyclase (or phospholipase C) signal transduction model (Stadel et al., 1980). The techniques dominating the field involved solubilization, purification and reconstitution of the components of this signaling complex, demonstrating the ability to restore most receptor-mediated function in an artificial lipid environment with these three components (Lefkowitz et al.,