

# G Protein-Coupled Receptor Heteromerization: A Role in Allosteric Modulation of Ligand Binding<sup>[S]</sup>

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## ABSTRACT

It is becoming increasingly recognized that G protein-coupled receptors physically interact. These interactions may provide a mechanism for allosteric modulation of receptor function. In this study, we examined this possibility by using an established model system of a receptor heteromer consisting of  $\mu$  and  $\delta$  opioid receptors. We examined the effect of a number of  $\mu$  receptor ligands on the binding equilibrium and association and dissociation kinetics of a radiolabeled  $\delta$  receptor agonist, [<sup>3</sup>H]deltorphan II. We also examined the effect of  $\delta$  receptor ligands on the binding equilibrium and association and disso-

ciation kinetics of a radiolabeled  $\mu$  receptor agonist, [<sup>3</sup>H][D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol]-enkephalin ([<sup>3</sup>H]DAMGO). We show that  $\mu$  receptor ligands are capable of allosterically enhancing  $\delta$  receptor radioligand binding and vice versa. Thus, there is strong positive cooperativity between the two receptor units with remarkable consequences for ligand pharmacology. We find that the data can be simulated by adapting an allosteric receptor model previously developed for small molecules, suggesting that the ligand-occupied protomers function as allosteric modulators of the partner receptor's activity.

## Introduction

G protein-coupled receptors (GPCRs) comprise one of the largest gene families in the mammalian genome that respond to a wide range of stimuli, including biogenic amines, amino acids, peptides, lipids, nucleosides, and large polypeptides. GPCRs are involved in a variety of biological processes, including neurotransmission, metabolism, and cellular differentiation, among others, and are therefore important targets for drug development (Rozenfeld et al., 2006; Kenakin and Miller, 2010). Many therapeutic agents target the orthosteric site of GPCRs (the site to which the endogenous ligand binds). These drugs either activate (agonists) or block (antagonists) receptor function. More recently, efforts have been made toward the identification of drugs that do not directly bind to the orthosteric site but are able to efficiently mod-

ulate GPCR function (Soudijn et al., 2004; Ma et al., 2009; Duvoisin et al., 2010). The advantage of this approach is the development of drugs that have fewer side effects and are better able to distinguish between GPCR subtypes.

A number of studies have shown that GPCRs can form dimers or oligomers (for the sake of simplicity, throughout the text, we will refer to oligomers as dimers; complexes consisting of two or more identical monomers, also known as protomers, as homomers; and complexes of two different protomers as heteromers). The existence of GPCR homomers and heteromers has been shown to occur in heterologous cells, in cell lines endogenously expressing receptors, in primary cell cultures, and in a few cases in intact tissues (for reviews, see Rios et al., 2001; Prinster et al., 2005; Rozenfeld and Devi, 2010b). In some cases, GPCR heteromerization has been shown to be essential for the formation of a functional receptor; the best known examples are GABA<sub>B</sub> and some taste and odorant receptors (White et al., 1998; Nelson et al., 2002; Neuhaus et al., 2005). In other cases, studies show that GPCR heteromerization leads to the modulation of the pharmacological, signaling, and trafficking properties of individual protomers (Rios et al., 2001; Prinster et al., 2005; Milligan, 2009).

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**ABBREVIATIONS:** GPCR, G protein-coupled receptor; OR, opioid receptor; TIPP $\psi$ , Tyr-Tic $\psi$ (CH<sub>2</sub>NH)-Phe-Phe; DAMGO, [D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol]-enkephalin; deltorphan II, Tyr-D-Ala-Phe-Glu-Val-Gly; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH<sub>2</sub>; BNTX, 7-benzylidenenaltrexone maleate; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH<sub>2</sub>; CHO, Chinese hamster ovary; ICI 174,864, N,N-diallyl-Tyr-Aib-Aib-Phe-Leu; SNC80, (+)-4-[( $\alpha$ R)- $\alpha$ -(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethylbenzamide; ANOVA, analysis of variance; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo-[1,2,3-d,e]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone.