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Allosteric modulators could be especially valuable in controlling receptors for which the design of orthosteric agonists or antagonists has been elusive.

Keynote review: **Allosterism in membrane receptors**

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Allosteric modulation of membrane receptors has been intensively studied in the past three decades and is now considered to be an important indirect mechanism for the control of receptor function. The allosteric site on the $GABA_A$ receptor is the target for the most widely prescribed sleep medicines, the benzodiazepines. Cinacalcet, an allosteric enhancer of the calcium-sensing receptor, is used to treat secondary hyperparathyroidism. Allosteric ligands might be especially valuable to control receptors for which the design of selective orthosteric agonists or antagonists has been elusive, such as muscarinic acetylcholine receptors.

Modulation of membrane receptor function is crucial for controlling cellular processes. An important mechanism of controlling receptor function is allosteric modulation, in which modulators bind to a regulatory site on a receptor distinct from the orthosteric site, which is the site of binding of the native ligand. Allosteric modulators induce conformational changes that profoundly influence the behavior of a membrane receptor in response to its native ligand [1–3]. There are positive, negative and neutral allosteric modulators of membrane receptors.

Christian Bohr [4] made the initial observation of the phenomenon of allostery. He carefully measured the oxygenation of hemoglobin and found that the binding curve was sigmoidal instead of hyperbolic, indicating cooperativity of binding of oxygen molecules to hemoglobin; he also found that carbon dioxide lowers oxygen affinity for hemoglobin. These properties make hemoglobin an efficient transporter of oxygen from the lungs to the tissues and of carbon dioxide from the tissues to the lungs.

Before the 1960s, hemoglobin was one of the few known examples of proteins that displayed allosteric properties. It was later demonstrated that many enzymes in bacteria and in higher organisms are subject to allosteric modulation. Researchers later discovered that in most allosteric proteins, indirect interactions between distinct specific binding sites account for the performance of their modulatory function [1]. An extension of the allosteric theory to membrane receptors was proposed in the late 1960s [3,5].

Although the history of allostery spanned over a century, it is still relevant to current research on protein function and signal transduction mechanisms [3]. Significant progress has been made in the investigation of allosteric modulation of protein action. The presence of allosteric sites

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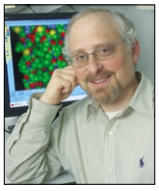

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1359-6446/06/$ – see front matter PII: S1359-6446(05)03689-5

on membrane receptors offers a novel pharmacological means of modulating receptor function. Allosteric sites have been found on ligand-gated ion channels (LGICs). An allosteric site on the γ-aminobutyric acid $(GABA)_A$ receptor provides the basis for the therapeutic effects of benzodiazepines [6]. Another germane example is the anticholinesterase inhibitor galantamine. In addition to its anticholinesterase activity, galantamine is also a positive allosteric modulator of α4β2 and α7 nicotinic receptors. Its successful use in the treatment of Alzheimer's disease might be an impetus for further development of more-potent and selective allosteric modulators [7]. Allosteric sites have also been demonstrated in G-protein-coupled receptors (GPCRs) [2]. Cinacalcet, a positive allosteric modulator of the calcium-sensing receptor (CaR), has been successfully used for the treatment of secondary hyperparathyroidism [8,9].

Therapy using receptor agonists is often prone to side effects owing to the widespread distribution of the target receptor in the body. An advantage of a positive allosteric modulator of a membrane receptor over its native, orthosteric activator is that, in principle, greater selectivity can be achieved. The positive allosteric modulator would enhance the action of the native agonist but might have no effect of its own on the unoccupied receptor. Thus, the agonist effect, which might be insufficient in a particular disease state, might be magnified through allosteric modulation. The higher subtype selectivity commonly exerted by allosteric modulators, and the fact that the allosteric action is ideally coupled to the simultaneous presence of the endogenous ligand, both help to prevent over-dosage compared with the administration of a conventional nonselective orthosteric agonist.

GPCRs

GPCRs, which constitute the largest family of cell-surface receptors, are major targets of drugs currently in clinical use. The GPCRs display the characteristic motif of seven transmembrane helices (TMs) and thus are also referred to as 7TM receptors. The 7TM proteins account for ~4% of the human genome. Mammalian GPCRs can be divided into three major subfamilies: class A, rhodopsin-like receptors; class B, secretin-like receptors; and class C, metabotropic glutamate-like and pheromone receptors. The rhodopsin-like receptors represent the predominant class of GPCRs. Members of a particular subfamily feature a substantial degree of amino acid homology, whereas different subfamilies demonstrate very low amino acid homology. Among the class A GPCRs, only the crystal structure of bovine rhodopsin has been determined [10].

The orthosteric ligand-binding site of a GPCR would be located either among TM helices or within the extracellular domain [3,10,11]. The third intracellular loop and carboxy-terminal end could interact with a G protein. Originally, a single receptor unit was thought to mediate the allosteric interactions between a modulator and the receptor. However, recent experiments suggest that the active forms of GPCRs can occur as GPCR dimers [12]. A dimerized nature of GPCRs is possibly associated with a diversification in their interactions with different G proteins, as well as in their desensitization and sequestration properties. It can also be argued that the functional unit of a GPCR is monomeric rather than dimeric or oligomeric [13]. Indeed, dimerization does not seem to be required for functional coupling of some GPCRs to G proteins but rather for its intracellular trafficking [14,15]. Thus, it is possible that, at least in some cases, the monomer is sufficient as a functional unit for some of the class A GPCRs but this might not be the case for other classes of GPCRs. For example, a heterodimer is essential for the $GABA_B$ receptor to function properly [16]. Taste receptors also exhibit this property [16]. Evidence suggests that the T1R1 and T1R3 taste receptors, which respond to sweet taste, combine as one functional unit [17].

An increasing percentage of GPCRs has been found to be modulated allosterically by various compounds. These receptors include all five subtypes of muscarinic receptors [18], at least three of the four subtypes of adenosine receptors [19], dopamine receptors [20], several class B GPCRs [21] and class C GPCRs [11]. The flexible nature of the interactions between the receptors and various allosteric modulators, combined with the potential for subtype selectivity, makes allosteric sites attractive for therapeutic intervention [2,22]. An allosteric modulator of CaR, cinacalcet, has been successfully administered for the treatment of secondary hyperparathyroidism [8].

A detailed mathematical analysis of the action of allosteric modulators of GPCRs has been described by Christopoulos [3], and Birdsall and Lazareno [18]. The allosteric ternary complex model (ATCM) features a term for the degree of cooperativity (α). The cooperativity factor $\alpha>1$ denotes a positive cooperativity; $\alpha<1$ results in negative cooperativity; neutral cooperativity occurs in cases where $\alpha = 1$. It should be noted that ATCM details the action of all allosteric modulators (not just enhancers) on the binding of orthosteric ligands to GPCRs but not on the signaling. The ATCM has been validated by direct binding measurements at an allosteric site on the muscarinic M2 receptor using an allosteric radioligand [23]. The model has recently been extended by Hall [24], Price *et al.* [25] and Ehlert [26] to accommodate the effects of allosteric modulators on efficacy. The effect of an allosteric modulator on agonist affinity and efficacy has been described in detail. A given allosteric modulator might have different effects on each of these parameters.

Class A GPCRs: rhodopsin-like receptors

Adenosine receptors

The A_1 adenosine receptor (AR) was the first subtype in the AR family for which selective orthosteric and allosteric ligands were developed. The aminobenzoylthiophene derivative 2-amino-4,5-dimethyl-3-thienyl-[3-trifluoromethylphenyl]methanone (PD81723; Figure 1) was the first allosteric enhancer in the GPCR field, as originally observed by Bruns and Fergus [27]. They found that PD81723 enhances the binding of agonist radioligand [^{3}H]cyclohexyladenosine to A_1 ARs and decreases the rate of dissociation of [^{3}H]cyclohexyladenosine from these receptors [27], suggesting that these compounds act at an allosteric site distinct from the orthosteric adenosine binding site to stabilize agonist–receptor–G-protein complexes. The aminobenzoylthiophenes were found to be highly selective for A_1 ARs, with little or no effect on the binding of agonists to other ARs or other GPCRs [27].

The behavior of A_1 AR enhancers has traditionally been complicated by both enhancement at low concentrations and inhibition at high concentrations [27]. Recently, several new series of 2-amino-3-benzoylthiophenes have been synthesized [28,29]. Several compounds proved to be superior to PD81723: they are more potent than PD81723 in enhancing the binding of A_1 AR agonist N^6-cyclopentyladenosine to the receptor and their antagonistic