Allosteric activation of the CaR by L-amino acids

Brian Kobilka*

Howard Hughes Medical Institute, Department of Molecular and Cellular Physiology and Division of Cardiovascular Medicine, Stanford University Medical School, Stanford CA 94305

G protein-coupled receptors (GPCRs) serve as cellular sensors for a broad range of stimulants including photons, odorants, hormones, neurotransmitters, and ions. The studies presented by Conigrave and colleagues (1) in this issue provide evidence that the cellular sensor for L-amino acids is also a GPCR. The extracellular calcium receptor (CaR), a member of the metabotropic glutamate receptor (mGluR) family of GPCRs, is responsible for maintaining Ca^{2+} homeostasis by regulating parathyroid hormone release and renal Ca^{2+} excretion. The investigators demonstrate that this receptor is also capable of detecting physiologic levels of specific L-amino acids. Thus, like the other members of this subfamily of GPCRs, the activity of the CaR can be modulated by structurally diverse agonists. mGluR subtypes 1α, 3, and 5 respond to both glutamate and extracellular Ca^{2+} (2), and the γ-aminobutyric acid type B ($GABA_B$) receptor response to GABA is enhanced by extracellular Ca^{2+} (3). The dual sensitivity of the CaR to both Ca^{2+} and specific L-amino acids has interesting implications for the coordinate regulation of Ca^{2+} homeostasis and amino acid metabolism. Moreover, the CaR is a rare example of a GPCR that is allosterically regulated by physiologic ligands, and it will be of great interest to learn the molecular mechanism of receptor activation.

The initial biochemical characterization of GPCR model systems (such as phototransduction and β-adrenergic receptor activation of adenylyl cyclase) led to the discovery of a relatively simple paradigm of GPCR signaling. The agonist-bound receptor activates a specific G protein heterotrimer, which dissociates into α- and $\beta\gamma$-subunits. The Gα- or the G$\beta\gamma$-subunit then modulates the activity of an effector. Although this paradigm still forms the core of GPCR signaling pathways, advances in this field over the past decade have shown that the signaling cascade for many GPCRs is considerably more complex and often interconnected with signaling pathways from other receptor systems. Cells often receive simultaneous activation from several extracellular factors that act on different GPCRs. For example, ATP, neuropeptide Y, and norepinephrine may all be released from sympathetic nerve terminals in the heart. Moreover, a given hormone or neurotransmitter may activate more than one target receptor on a given cell. Cardiac myocytes express β_2-, β_1-, and α_1-adrenoceptors, which are all activated by norepinephrine. Finally, many receptors can activate more than one family of G proteins as well as modulate the activity of growth factor pathways once thought to be the domain of receptor tyrosine kinases (4). There is a growing body of evidence that the promiscuous nature of GPCR signaling is restrained by proteins that connect signaling molecules with each other or with cytoskeletal elements, thereby restricting access of receptors to the appropriate signaling partners (4). The cell-type-specific signaling of GPCRs is therefore likely to be determined by the specific receptors, G proteins, and effector molecules expressed in the cell, as well as by the scaffold proteins that physically connect components of the signaling cascade.

Integration of signals resulting from the activation of cells by more than one GPCR agonist can be accomplished in several ways. Stimulation of a receptor for agonist A may modify the expression of genes for proteins in the signaling pathway of the receptor for agonist B or may lead to the phosphorylation of the receptor for B, the G protein, or the effector activated by this receptor. Phosphorylation and dephosphorylation of receptors has been shown to play an important role in GPCR function, and recent studies suggest that receptor phosphorylation may even change its G protein-coupling specificity (5). The observation by Conigrave and colleagues (1) that the CaR can be activated allosterically by specific L-amino acids as well as Ca^{2+} provides yet another mechanism by which information in the form of a complex mixture of hormones, neurotransmitters, and metabolites can be integrated by cells.

There are five families of GPCRs (A, B, C, D, and E) based on sequence homology. GABA, mGluR, and the CaR belong to family C. For receptors in the largest family (rhodopsin family or family A), the location of the ligand-binding domain roughly correlates with the size of the ligand (6). Small agonists bind within the transmembrane (TM) domains. Peptide agonists bind primarily to extracellular sequences connecting the TM domains. The major determinants for the binding of large protein hormones such as luteinizing hormone and follicle-stimulating hormone are in the large extracellular amino-terminal domain. For small catecholamine agonists, it has been shown that binding leads to changes in the relative orientation of TM domains that form the catecholamine-binding pocket (7). In contrast to small molecular mass agonist binding to GPCRs in family A, the major determinants for agonist binding to members of the GPCR family C are found within the large extracellular amino terminus characteristic of this family of receptors. The mechanism by which the binding of these small agonists (Ca^{2+}, glutamate, and γ-amino butyric acid) to the amino-terminal domain leads to conformational changes in the TM domains has yet to be determined. It is possible that conformational changes induced in the large amino-terminal domain by agonist binding are transmitted to the TM domains by structural contacts between the amino terminus and extracellular loops connecting the TM domains.

Allosteric modulation of GPCRs has been characterized most extensively for muscarinic receptors (8). Both negative and positive allosteric regulation of antagonist binding to muscarinic receptors has been observed experimentally. Allosteric regulation can be demonstrated in all five subtypes of muscarinic receptors, but the m2 receptor seems to be the most sensitive. Mutagenesis and chimeric receptor studies suggest that the allosteric binding site is close to the orthosteric binding site, which lies within the TM domains. Nevertheless, unlike the effect of L-amino acids on CaR activation, allosteric enhancement of agonist binding or activation has not been observed in muscarinic receptors. Although a number of natural compounds have been identified as allosteric modulators of muscarinic function, none have been shown to be physiologically important (8). Sodium has been

See companion article on page 4814.

*E-mail: kobilka@cmgm.stanford.edu.

shown to be an allosteric modulator of agonist binding for a number of GPCRs. In the case of the α_2-adrenergic receptors, the amino acid responsible for this sodium sensitivity is found on the cytoplasmic side of the second TM domain (9), and there is experimental evidence that the receptor is responsive to fluctuations of intracellular sodium ions (10).

The allosteric binding site for L-amino acids on the CaR has yet to be determined; however, by analogy with the mGluR receptor and the $GABA_B$ receptor, it might be predicted that L-amino acids bind within the large extracellular amino terminus. Thus, for the mGluR, the Ca^{2+} binding site and the glutamate binding site have both been localized to the amino terminus by site-directed mutagenesis (2). However, it is also likely that TM domains form the L-amino acid binding site. The ligands for the receptors for catecholamines, dopamine, and serotonin have been shown to bind within the TM domains (6). These ligands are all derived from aromatic amino acids. Moreover, several activating mutations of the CaR that are responsible for increased sensitivity to extracellular Ca^{2+} are found at the borders of TM domains (11, 12). Thus, the binding of specific amino acids within the TM domains may disrupt some of the intramolecular interactions responsible for maintaining the receptor in an inactive state, thereby reducing the energy barrier for activation by the amino terminus bound to Ca^{2+}.

Although it was once generally accepted that GPCRs function as monomers, there is a growing body of evidence that receptor dimers form and play a role in receptor function. It is of interest that homodimers have been observed for mGluRs (13) and CaRs (14). More interesting is the observation that the $GABA_BR1$ receptor must form a heterodimer with the $GABA_BR2$ receptor to be functional (15–17). Of interest, muscarinic receptors have also been shown to form homodimers (18). Therefore, one must consider a possible role for receptor dimers in allosteric modulation of receptor activity. It is possible that one of the receptors in the dimer serves as the orthosteric binding site, while the other receptor serves as the allosteric binding site.

The coupling of protein ingestion to the release of digestive enzymes and insulin and to the promotion of gastric motility requires a cellular sensor for amino acids. The sensitivity of the CaR to specific amino acids and its expression in stomach suggest that the CaR is a physiologic amino acid receptor. However, specific L-amino acids serve as positive allosteric regulators of the CaR in the presence of Ca^{2+} but are inactive in the absence of Ca^{2+} (1). As discussed in the article by Conigrave and colleagues (1), there are several physiologic states that lead to concomitant elevations in both Ca^{2+} and L-amino acids. Nevertheless, the normal Ca^{2+} concentration in extracellular fluid may be sufficient to permit responses to elevations of amino acids in the absence of fluctuations of extracellular Ca^{2+}. Moreover, it remains a possibility that L-amino acids may act as independent agonists for the CaR when it is coupled to another signaling pathway or when it is expressed in a specific differentiated cell *in vivo*. The finding that the P2Y receptor (a receptor for ATP) may be allosterically activated by physiologic levels of branched chain amino acids (1) suggests that multiple GPCRs may play a role in metabolic responses to amino acids. Clues to the physiologic importance of the CaR as an amino acid sensor may be obtained by careful study of patients with inherited forms of hypoparathyroidism and hyperparathyroidism caused by mutations in the CaR. It will be of interest to determine how these mutations affect the response of the receptor to amino acids in *in vitro* signaling assays and *in vivo*. It may be possible to identify mutations that selectively alter Ca^{2+} signaling or selectively modify the response of the receptor to amino acids. Closer examination of patients may reveal physiologic consequences directly related to alterations in the ability of the receptor to detect L-amino acids.

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