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RESEARCH HIGHLIGHT

$GABA_B$ receptor pas de deux: insights from high-resolution structures

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The $GABA_B$ receptors are heterodimeric class C G protein-coupled receptors (GPCRs) that mediate the inhibitory neurotransmission in the central nervous system and they are drug targets for alcoholism and pain control. In a recent paper published in *Cell Research,* Mao et al. reported the first full-length $GABA_B$ heterodimer structures in both inactive and active states, providing a framework for understanding the activation of class C GPCRs and a template for designing allosteric modulators targeting the dimeric interface of the $GABA_B$ receptors.

The metabotropic gamma-aminobutyric acid (GABA) type B ($GABA_B$) receptors belong to class C G protein-coupled receptors (GPCRs). They function as an obligatory heterodimer of two subunits, $GABA_{B1}$ (GB1) and $GABA_{B2}$ (GB2). GB1 subunit binds orthosteric ligands, while GB2 couples with the Gi/o protein. The $GABA_B$ receptors possess a large extracellular domain containing an orthosteric binding pocket named Venus flytrap (VFT) domain. The VFT domain is connected to the seven-transmembrane domain (7TMD) through a shorter stalk region, which lacks the cysteine-rich domain (CRD) present in other class C GPCRs.[1,2] Considerable efforts have been devoted to clarifying the molecular mechanism of $GABA_B$ receptor activation. The structures of $GABA_B$ receptor heterodimeric VFT domain in apo, antagonist- and agonist-bound states reveal the open and close conformations of VFT domain.[3] The recently reported structures of full-length metabotropic glutamate receptor 5 (mGlu5), another representative class C GPCR, in apo and agonist-bound states provide a putative framework for class C GPCR activation.[4] However, the activation mechanism of $GABA_B$ remains elusive due to a lack of high-resolution structures of full-length $GABA_B$ receptors.

The $GABA_B$ receptors mediate transduction of slow and prolonged inhibitory signals in the central nervous system and represent an attractive drug target for alcoholism and pain control. The orthosteric agonist baclofen has been approved for the treatment of muscle spasticity. A variety of allosteric modulators have exhibited similar preclinical therapeutic effects. Furthermore, they do not display the side effects of sedation and muscle relaxation associated with baclofen, making them promising candidates.[5,6] Rational drug design targeting the $GABA_B$ receptors will benefit from high-resolution structures of $GABA_B$ receptors bound to agonists and allosteric modulators.

The paper by Mao et al.[7] reported the first set of structures of the full-length human heterodimeric $GABA_B$ receptors, at a resolution range of 2.8-3.0 Å, in the antagonist-bound inactive state and in the active state complexed with a agonist, a positive allosteric modulator (PAM) and an inhibitory G protein heterotrimer (Gi1) (Fig. 1). These structures are the highest resolution structures of class C GPCRs reported so far and reveal a possible overall activation mode similar to the other class C GPCRs: binding of agonist induces the compaction of the large extracellular VFT domain from two subunits and relays the signal to the 7TMD through the stalk region. Consequently, each monomer in the TMD dimer is rotated along each other to form a new TM6-mediated interface, which appears to be the hallmark of class C GPCR activation.[2,4]

Although the $GABA_B$ receptors share a common activation mode, several unique structural features of the $GABA_B$ receptors are involved in the receptor activation, indicating a receptor-specific activation mechanism. The first and most obvious feature is the asymmetric activation of the $GABA_B$ receptors. Compared to GB1, which shows negligible conformation change after baclofen binding to VFT of GB1, substantial conformation changes happen at the stalk region and TM3/4/5-ICL3 at the cytoplasmic part of the GB2, leading to the coupling of Gi1 protein predominantly to GB2 subunit. Interestingly, lacking CRD in the $GABA_B$ receptors may make the stalk region relatively shorter and ECL2 regions more rigid, thus allowing the receptors to transduce conformation changes from VFT to 7TMD.[8] Secondly, several putative cholesterols exist at the TMD interface of the inactive $GABA_B$ receptors, indicating a possible involvement of these cholesterols in maintaining the inactive state of the receptors. Additionally, in each TMD from both the inactive and active $GABA_B$ receptors, two-chained phospholipids are found to occupy a site that corresponds to the orthosteric site in class A GPCRs. The presence of phospholipids was also identified in the structure of the inactive full-length $GABA_B$ receptors released in a paper published online.[8] These phospholipids are conceived to associate with ECL2 and participate in the stabilization of stalk region, subsequently propagating signals from VFTs to TMDs.[8] Lastly, the TM6-TM6 interface of active $GABA_B$ heterodimer is stabilized by BHFF, a potent and selective PAM. Noteworthily, the PAM (+)-BHFF was well fit into the density, supporting the fact that the (+)-BHFF shows a higher activity compared to rac-BHFF. So far, the allosteric modulators of class C GPCRs were reported to share a common site overlapping with the orthosteric binding pocket in class A GPCRs. Therefore, this binding site at the interface of TMDs from GB1 and GB2 subunits represents a novel putative allosteric pocket, which was further identified by the structure of PAM GS39783-bound $GABA_B$ heterodimer.[9] The allosteric binding

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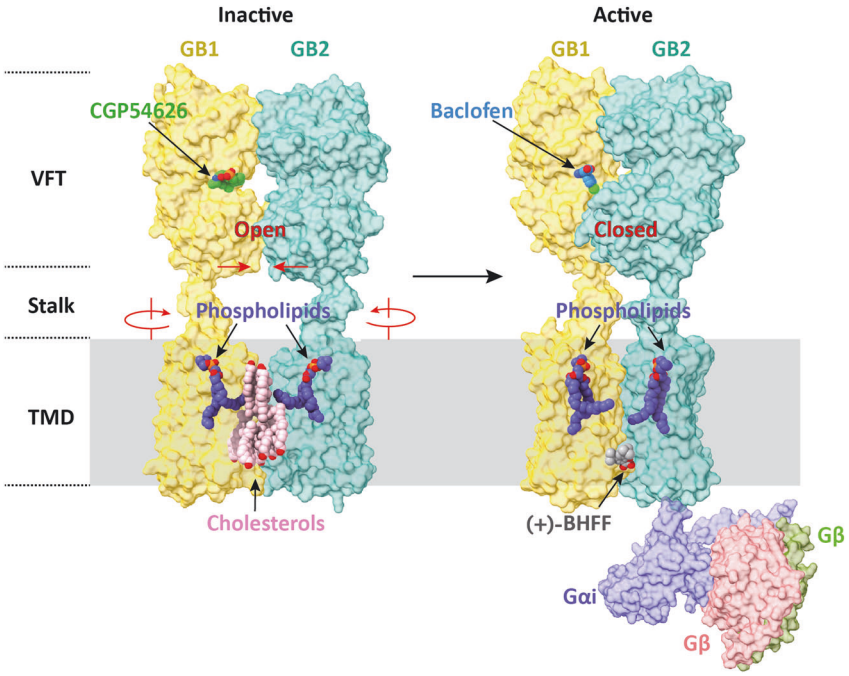

Fig. 1 Surface illustrations of the structure models of the inactive and active $GABA_B$ receptors. Left panel, the structure of the inactive $GABA_B$ receptors bound to antagonist CGP54626. VFT exists in an open state. Conformation changes involved in $GABA_B$ receptor activation are indicated by red arrows. Right panel, the structure of active $GABA_B$ receptors bound to agonist baclofen and PAM (+)-BHFF. Binding of agonists stabilizes VFT in the closed state. The putative model of $GABA_B$ receptors-Gi coupling is indicated. Cholesterols and phospholipids are shown as spheres. The PAM (+)-BHFF binds at the interface of TMDs from GB1 and GB2 subunits.

pocket may provide a new structural template for designing PAMs against $GABA_B$ receptors.

Furthermore, for the first time, Mao et al. provide three structural models for $GABA_B$ receptors-Gi coupling. For class A GPCRs, the pronounced outward movement of TM6 at the cytoplasmic end opens a large cytoplasmic cavity more accessible to a G protein.[10] Intriguingly, the small-amplitude arrangements of TM3/4/5 and ICL3 of the $GABA_B$ receptors create a shallow binding site for the Gi1 protein, translating to a ~8 Å downward shift of α5 helix of Gi1 relative to that of the class A GPCR-G protein complexes. This structural feature provides a putative explanation for the heterogeneity of $GABA_B$ receptors-Gi coupling. A high-resolution structure of the $GABA_B$ receptors bound to Gi protein is still awaited to uncover the mechanism of class C GPCR coupling to a G protein.

Together, the first high-resolution cryo-EM structures of the inactive and active $GABA_B$ receptors presented by Mao et al. significantly extends our understanding of the structure and function of class C GPCRs. These findings not only provide a putative structural explanation of the $GABA_B$ receptor activation mechanism, but also offer a basic template for rational design of allosteric modulator drugs. Going forward, it will be important to learn how the $GABA_B$ receptors interact with Gi protein at high resolution, how the G protein coupling mechanism is generalizable amongst class C GPCRs, and how cholesterols and phospholipids regulate class C GPCRs in different activity states. More high-resolution structures of class C GPCRs with G proteins are expected to address these important questions.

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