RESEARCH HIGHLIGHT

Steering G protein activation by mGlu heterodimer

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Heterodimerization of metabotropic glutamate receptors (mGlus) generates functional units that modulate the synapse activity, and displays strong therapeutic potential for treating brain disorders and psychiatry diseases. Here, Wang et al. solved the cryo-EM structures of $mGlu_2$–$mGlu_3$, and $mGlu_2$–$mGlu_4$ heterodimers in various conformational states, revealing the role of each subunit in the asymmetric signaling of mGlu heterodimers and the molecular basis of their allosteric modulation, and giving a rationale to understand which subunit activates the G protein.

The metabotropic glutamate receptors (mGlus) are G protein-coupled receptors broadly expressed in the central nervous system and play important roles in neurobiological function. There are eight subtypes of mGlu receptors that form homo- and heterodimers, and the dimerization is essential to their function. mGlu heterodimers were reported,[1] including $mGlu_1$–$mGlu_5$, $mGlu_2$–$mGlu_4$, $mGlu_2$–$mGlu_3$, $mGlu_2$–$mGlu_7$ mainly. The existence of $mGlu_2$–$mGlu_4$ heterodimer in mouse brain was recently reported, highlighting their physiological importance and potential role in brain functions and disorders.[2,3] The mGlu heterodimers offer new opportunities for tuning the synaptic activity. However, the molecular basis of their dimerization and asymmetric activation has remained unresolved.

mGlu homodimers are activated by glutamate binding in the Venus flytrap domains (VFTs) stabilizing the closed state (c) and active (A) conformation of the VFT dimer. These first events trigger the movement of the cysteine-rich domains (CDRs) and conserved heptahelical transmembrane domains (7TMs) promoting inter-subunit molecular contacts and stabilizing the active state of the receptor.[4–6] Conversely, antagonist binding maintains the VFTs in an open state (o) and inactive (R) conformation of the dimer, preventing the reorganization of both subunits and inhibiting the receptor activation.[7] The twelve cryo-EM structures reported in Wang et al.[8] illustrate the complexity and diversity of the $mGlu_2$–$mGlu_3$ and $mGlu_2$–$mGlu_4$ dimerization modes, positive allosteric modulation, signal transduction and the multiple conformations populating the mGlu heterodimer conformational landscape (Roo, Rco, Acc with or without G protein).

The structures of the $mGlu_2$–$mGlu_3$ heterodimer obtained in combination with the antagonist LY341495, the $mGlu_2$-selective negative allosteric modulator (NAM) NAM563 and the $mGlu_3$-selective NAM LY2389575 represent diverse inactive states of $mGlu_2$–$mGlu_3$. All the structures adopt an Roo conformation in the VFTs but with differences in the 7TM dimerization modes: mode I that is stabilized by cholesterol, modes II and III in which the 7TMs are closer but using different protein–protein interfaces.

$mGlu_2$–$mGlu_3$ was also captured in an Rco intermediate inactive state, in dimerization mode I, with NAM563 bound to $mGlu_2$ 7TM but with an empty and open VFT, whereas $mGlu_3$ VFT is stabilized in closed state by glutamate. The higher glutamate affinity of $mGlu_3$ likely accounts for such intermediate inactive Rco state. A similar conformation is also reported in the presence of glutamate and $mGlu_2$ positive allosteric modulator (PAM) JNJ-40411813, but a glutamate molecule is bound to the $mGlu_2$ VFT that remains open. Together, such subtle differences in the Rco intermediate inactive state highlights part of the first sequential reorganization of the VFTs towards the active state of the receptor (Fig. 1).

The transition from intermediate inactive (Rco) to intermediate active state (Acc without G protein) of the $mGlu_2$–$mGlu_3$ and $mGlu_2$–$mGlu_4$ heterodimers brings the CRDs in close proximity and the reorganization of the 7TM dimerization mode, as previously described for mGlu homodimer.[4–6] However, the 7TM interactions are shifted to an asymmetrical interface with some differences between the two heterodimers. The $mGlu_3$ subunit is tilted away from the $mGlu_2$ subunit whereas the $mGlu_4$ is rotating anticlockwise by 20°. By combining a mutation that blocks the G protein binding (F765S, in $mGlu_3$) and mutations that prevent the binding of glutamate (YADA), the functional analysis provides the final demonstration that the closure of $mGlu_3$ is likely sufficient for activating the G protein via $mGlu_2$ 7TM, in a *trans*-activation mechanism similar to the signal transduction mechanism of the heterodimeric $GABA_B$ receptor, a member of the class C GPCRs.[9]

The next question is what drives G protein activation by one protomer over the other in mGlu heterodimers? Wang et al. report the G protein-coupled structures of $mGlu_2$–$mGlu_3$ in complex with $mGlu_2$ PAM JNJ40411813 and the $mGlu_2$–$mGlu_4$ in complex with JNJ40411813 and $mGlu_4$ PAM ADX88178. While in the case of the $mGlu_2$–$mGlu_4$ heterodimer, $mGlu_4$ was previously identified as the subunit coupling to the G protein,[10] the G protein is now bound to the $mGlu_2$ subunit, highlighting the ability of $mGlu_2$ to activate the G protein in the heterodimer. The main parameter driving the identity of the subunit recruiting the G protein is suggested to be the respective stability of the 7TM inactive state, with the less stable conformation favoring G protein binding and activation. In that respect, the conserved tryptophan $W^{6.50}$ in TM6 happens to be central in this mechanism and for controlling the equilibrium between inactive and active states of the helical bundle. The movement of $W^{6.50}$, homologous position of the so-called toggle switch in class A GPCRs, is more constrained in $mGlu_2$ ($W773^{6.50}$), due to a hydrogen bond established with $N735^{5.47}$. In $mGlu_4$, however, the position 5.47 (S760) is occupied by a serine that cannot form a hydrogen bond with $W798^{6.50}$, making $mGlu_4$ more prone to activating the G protein. A similar but reverse situation takes place at the $mGlu_2$–$mGlu_3$ heterodimer, where position 5.47 in $mGlu_3$ is an aspartate which is a better hydrogen-bond acceptor

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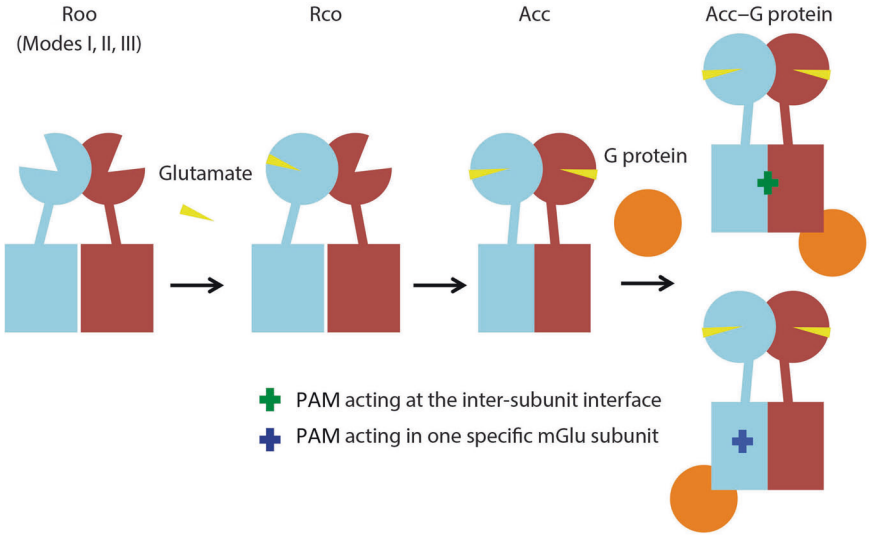

Fig. 1 Schematic model showing allosteric modulation of mGlu heterodimers and G protein activation. Inactive state Roo displays some diversity with different dimerization modes (modes I, II, III). Glutamate binding induces the closure of one VFT that likely drives the rearrangement of the heterodimer quaternary structure (Rco state). In the intermediate active state structure (Acc), and in the presence of G protein (Acc–G protein), both VFTs bind glutamate and are in closed state but with some differences at the helical bundle interface. The selection of the mGlu subunit that activates the G protein depends on the stability of each subunit in the inactive state and can be pharmacologically modulated by drugs acting either in one of the 7TMs (blue) or at their molecular interface (green).

compared to the asparagine in $mGlu_2$, making $mGlu_3$ 7TM inactive state more stable and explaining the G protein activation by $mGlu_2$.

It is however possible to pharmacologically modulate G protein coupling in $mGlu_2$–$mGlu_3$/$mGlu_4$ heterodimers. Destabilizing the $mGlu_2$ inactive state using a PAM switches the G protein activation by from $mGlu_4$ to $mGlu_2$; or contrarily, increasing the stability of $mGlu_2$ 7TM inactive state using the $mGlu_2$-selective NAM563, partially recovers some G protein activation by $mGlu_3$. Moreover, allosteric modulators may also act on the ternary structure of the complex as illustrated by the $mGlu_4$ PAM ADX88178 that unexpectedly targets the interface between $mGlu_2$ 7TM and $mGlu_4$ 7TM by interacting with both subunits, and triggers G protein activation through $mGlu_4$ only. Overall, it becomes clear that the allosteric modulation can be a powerful strategy for taking control of a given mGlu subunit and to orientate the signal outcome of the heterodimer. GPCR function can be twisted by molecules that exploit unusual large diversity of binding sites.

The study by Wang et al. is a great example of the multiple conformations of the mGlu heterodimers in Roo, Rco, Acc (G protein-free), Acc (G protein-bound) states, which sequentially lead to G protein activation (Fig. 1). The activation mechanism of the $mGlu_2$–$mGlu_4$ and $mGlu_2$–$mGlu_3$ heterodimers opens great opportunities for subunit selectivity, providing unique but specific signal transduction signature of a given heterodimer.

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ADDITIONAL INFORMATION

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