Mechanisms of Orthosteric Agonist and Allosteric Modulator Activation of $mGlu_7$

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Abstract

Metabotropic glutamate receptor 7 ($mGlu_7$) is a dimeric, group III metabotropic glutamate (mGlu) receptor. It is a G protein-coupled receptor that acts to modulate neurotransmission across many brain structures. $mGlu_7$ is most highly expressed presynaptically in neurons and is widely distributed in the central nervous system (CNS) and body. Mutations, deletions, or decreases in $mGlu_7$ result in symptoms and phenotypes of neurodevelopmental disorders in humans and mice[1], including Rett syndrome. We are focused on developing new ligands that interact with $mGlu_7$ to understand the therapeutic potential of the receptor in various CNS disorders.

We are using two *in vitro* molecular pharmacology assays to test newly developed compounds for activity at $mGlu_7$. However, these and other *in vitro* studies show that the orthosteric ligand, glutamate, has low affinity for $mGlu_7$ which limits its ability to be routinely used for drug screening. An alternative agonist, L-AP4, is a synthetic compound that, while not produced naturally *in vivo*, activates $mGlu_7$ with higher affinity than glutamate. Our preliminary research indicates, however, that there are differences in the profiles of positive allosteric modulators (PAMs) when assessed using glutamate versus L-AP4. This difference creates a challenge for the development of $mGlu_7$-selective PAMs, and the focus of our current studies is to understand the difference between these two agonists in activating the $mGlu_7$ receptor.

Previous literature shows that some mGlu receptors demonstrate partial activity when only one agonist binding site is occupied and full activity when binding to both agonist binding sites occurs within an mGlu receptor dimer[2]. Therefore, we hypothesize that glutamate and L-AP4 activate $mGlu_7$ either via a different number of agonist binding sites or a different number of effector (G-protein) binding sites. Our current studies are designed to differentiate between these two possibilities.

$mGlu_7$ responds differently to Glutamate and L-AP4

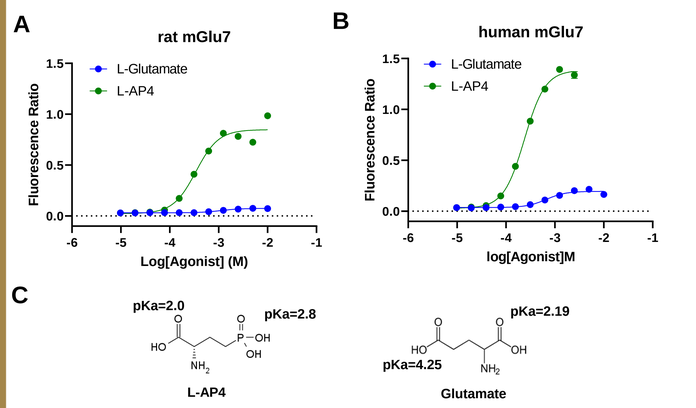

Figure 1. Low affinity and functional potency of glutamate at $mGlu_7$ necessitates an alternative agonist for functional drug screening. A. Concentration-response curve (CRC) of wild-type (WT) rat $mGlu_7$ with different agonists in HEK Gα15 cells as measured using a calcium mobilization assay. B. CRC of different agonists at WT human $mGlu_7$. C. Chemical structure of L-AP4 and Glutamate

References

1. Fisher, N. M., Seto, M., Lindsley, C. W. & Niswender, C. M. Metabotropic Glutamate Receptor 7: A New Therapeutic Target in Neurodevelopmental Disorders. *Front. Mol. Neurosci.* **11**, 1–14 (2018).
2. Kniazeff, J. *et al.* Closed state of both binding domains of homodimeric mGlu receptors is required for full activity. *Nat. Struct. Mol. Biol.* **11**, 706–713 (2004).
3. Margeta-Mitrovic, M., Jan, Y. N. & Jan, L. Y. Function of GB1 and GB2 subunits in G protein coupling of GABAB receptors. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 14649–14654 (2001).

Heterodimer restriction of mGlu7 subunits

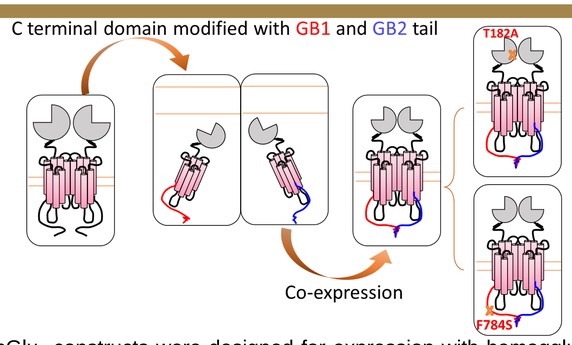

Figure 2. $mGlu_7$ constructs were designed for expression with hemagglutinin (HA) tags on the N terminus. The C terminal domains of the receptors were modified to contain tails derived from the two $GABA_B$ receptor subunits, GB1 and GB2; this strategy permits only heterodimers with this two subunit combination to traffic to the cell surface[3]. Individual $mGlu_7$ receptor subunits were then mutated at either the agonist binding site (T182A) or the G-protein activation site (F784S).

Modified $mGlu_7$ dimers are expressed on the cell surface

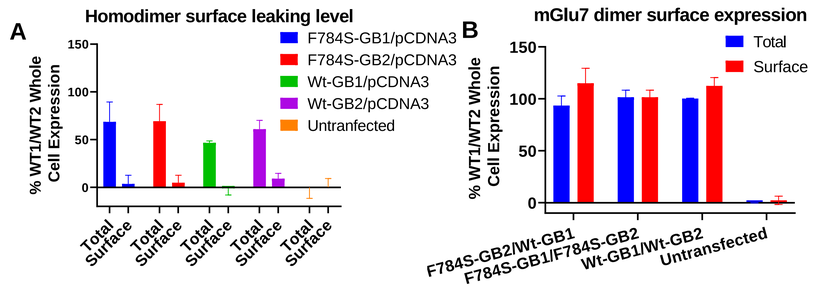

Figure 3. Evaluation of total and surface expression of $mGlu_7$ by on cell ELISA. A. When expressed alone, $mGlu_7$ receptors with either a $GABA_{B1}$ or $GABA_{B2}$ tails are expressed in cells but do not travel to the surface by themselves (N=1). B. When GB1 and GB2 tails are expressed together, dimers traffic efficiently to the cell surface (N=3).

Glutamate and L-AP4 activate G-protein binding sites differently

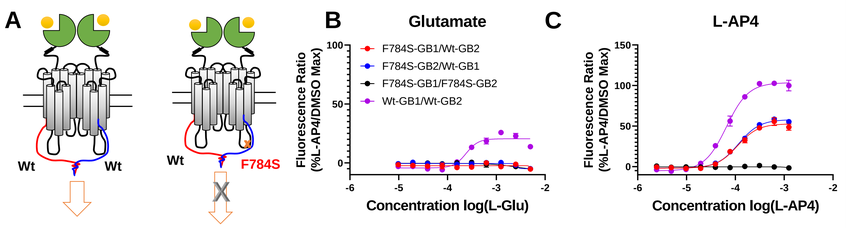

Figure 4. A. Schematic of $mGlu_7$ dimer activation both as a wild-type (WT) receptor tagged with GB1 (red) and GB2 (blue) tails and with an F784S mutation to disrupt G protein coupling in the GB2 subunit. Activation of various $mGlu_7$ dimers with glutamate (B) or L-AP4 (C). While L-AP4 is able to activate a receptor in which one G protein binding site has been eliminate, glutamate can not (N=1).

PAMs differentially affect receptor signaling with distinct agonists

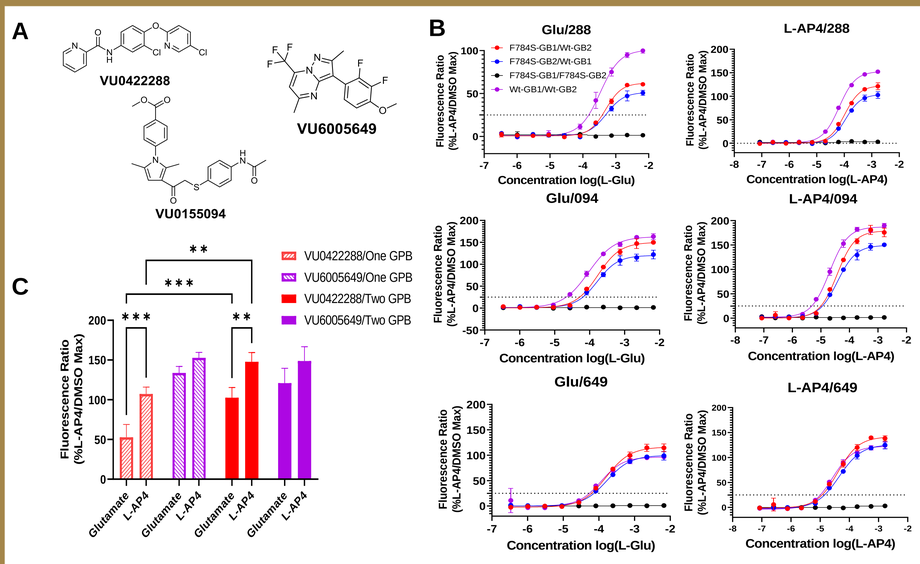

Figure 5. PAMs differentially affect G-protein activation. A. Structure of positive allosteric modulators (PAMs) used in these studies. VU288 and VU094 are group III mGlu PAMs and VU649 is $mGlu_{7/8}$ PAM. B. CRC of different $mGlu_7$ dimer responses to glutamate and L-AP4. C. The maximal responses of VU288 and VU649 from Ca assays with different agonists exhibit significant differences. N=4, 2-way ANOVA with Turkey post-test , **p<0.01, ***p<0.001, ****p<0.0001

Table. 1 LogEC50 from Ca assays with different agonists N=3

Dimers	DMSO		VU0422288		VU0155094		VU6005649	
F784S-GB1/Wt-GB2	N/A	-3.61±0.14	-3.35±0.08	-4.01±0.05	-3.80±0.04	-4.53±0.19	-3.79±0.01	-4.46±0.18
F784S-GB2/Wt-GB1	N/A	-3.62±0.10	-3.34±0.10	-4.00±0.06	-3.82±0.04	-4.52±0.19	-3.78±0.03	-4.43±0.17
F784S-GB1/F784S-GB2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Wt-GB1/Wt-GB2	-3.14±0.09	-3.81±0.09	-3.54±0.06	-4.24±0.09	-4.04±0.02	-4.76±0.12	-3.98±0.08	-4.63±0.08
Agonists	Glutamate	L-AP4	Glutamate	L-AP4	Glutamate	L-AP4	Glutamate	L-AP4

Discussion and future plans

1. Different agonists activate $mGlu_7$ distinctly based on the number of intact G-protein activation sites.
2. Glutamate activates $mGlu_7$ only when both G-protein binding sites are functional, while L-AP4 activates the receptor partially with one G-protein binding site.
3. The PAMs also show differential effects on signaling, with some PAMs requiring two G-protein binding sites to induce full activation while others only require one.

Studies with glutamate binding-site mutations (T182A) are currently underway, as well as studies regarding how each $mGlu_7$ protomer is activated within heterodimeric receptors.

Acknowledgements

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