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Mechanism of Orthosteric Agonists and Allosteric Modulators for mGlu7 Activation

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Introduction: Metabotropic glutamate receptor 7 (mGlu7) 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. mGlu7 is most highly expressed presynaptically in neurons and is widely distributed in the central nervous system (CNS) and body. Mutations, deletions, or decreases in mGlu7 result in symptoms and phenotypes of neurodevelopmental disorders in humans and mice1, including Rett syndrome. We are focused on developing new ligands that interact with mGlu7 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 mGlu7. However, these and other in vitro studies show that the orthosteric ligand, glutamate, has low affinity for mGlu7 that 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 naturally, activates mGlu7 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 mGlu7-selective PAMs, and the focus of our current studies is to understand the difference between these two agonists in activating the mGlu7 receptor. Previous literature shows that some mGlu receptors show 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 dimer2. Therefore, we hypothesize that glutamate and L-AP4 activate mGlu7 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.

Methods: mGlu7 constructs were designed for expression with HA tags on the N terminus. The C terminal domains of the receptors were modified to contain tails derived from the two GABAB receptor subunits, GB1 and GB2, a strategy that permits only the heterodimers with this two subunit combination to traffic to the cell surface3. Individual mGlu7 receptor subunits were then mutated at either the agonist binding site (T182A) or the G-protein activation site (F784S) through site- direct mutagenesis. Whole cell and surface expression levels were measured using Western blotting. Receptor activity was evaluated using a calcium assay.

Results: Tagged mGlu7 receptors were expressed in the cell, with only the combination of the GB1/GB2 subunits trafficking to the cell surface. We found that both G-protein binding sites were required for any glutamate-dependent activation. L-AP4, however, could partially activate the dimer with one functioning G-protein binding site, and two G-protein binding sites fully activated mGlu7 with L-AP4. The PAM VU0422288 partially potentiated mGlu7 with only one functional G-protein binding site and required two G-protein binding sites to be fully potentiated. In contrast, other PAMs (VU0155094 and VU6005469) could fully potentiate mGlu7 activity with one functioning G-protein binding sites . Studies with glutamate binding-site mutations are also currently underway.

Discussion: Our data suggest that different agonists activate mGlu7 distinctly based on the number of intact G-protein activation sites. Glutamate activates mGlu7 only when both G-protein binding sites are functional, while L-AP4 activates the receptor partially with one G-protein binding site. The PAMs also show different binding mechanisms, with some PAMs requiring two G-protein binding sites to be fully activated while others only require one.

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