A Novel Binding Assay for Metabotropic Glutamate Receptors Using [^{3}H] L-Quisqualic Acid and Recombinant Receptors

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We established a methodology to analyze radioligand binding to the recombinant type 1a metabotropic glutamate receptor (mGluR1a). A full-length cDNA encoding mGluR1a, which was isolated from a λ gt 11 cDNA library of human cerebellar origin, was expressed in a baculovirus/Sf9 insect cell system. Membrane fractions with recombinant receptor expression were analyzed for the binding of [^{3}H]L-quisqualic acid (L-QA), which is known to be a potent agonist of mGluR1a. Efficient binding of the radioligand to the human receptor was observed in a saturable manner, giving an apparent Kd= 0.091 μM. [^{3}H]L-QA bound to the human mGluR1a was displaced by known ligands such as L-QA, L-Glu, t-ACPD ((±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid) with IC_{50}s = 0.056, 0.97 and 4.0 μM, respectively. MCPG (α-methyl-4-carboxyphenylglycine) displaced the radioligand binding with lower potency. Using this binding protocol, we then evaluated the ligand ability of synthetic dipeptides. Among peptides tested, only Glu-containing dipeptides inhibited the radioligand binding, *e.g.* IC_{50} of L-Met-L-Glu was 4.3 μM. When phosphatidyl inositol turnover was assayed in mGluR1a-expressing CHO cells, L-Met-L-Glu was partially agonistic. We further expanded this [^{3}H]L-QA binding protocol to type 5a mGluR, another member of group I mGluRs, as well as to AMPA receptor, a member of ionotropic glutamate receptors, since L-QA is also known to be a potent ligand for these receptors. Data shown here will provide a novel system not only to search for ligands for the glutamate receptors, but also to biochemically analyze the interaction modes between glutamate receptors and their ligands.