Glutamate, the excitatory neurotransmitter of the central nervous system (CNS), has many physiological functions in the nervous system, including modulation of neuronal excitability, synaptic transmission, learning and memory, proliferation, and survival. These functions result mainly from its interactions with ionotropic and G protein-coupled metabotropic glutamate receptors (mGluRs).

mGluRs, a subset of the eight known mGluR subtypes, is present in many brain regions such as hippocampus, cortex, and olfactory system. The activation of mGluRs under pathologic conditions has been reported to be associated with psychiatric and neurodegenerative diseases, including Parkinson's disease, depression, schizophrenia, pain, and others (5). In contrast to these roles, mGluRs is a potential target for treatment of multiple CNS disorders. However, the highly conserved glutamate binding sites across mGluR subtypes remain difficult for highly selective agonists and antagonists.

An important alternative strategy is to develop highly selective modulators of mGluRs by measuring intracellular Ca2+ mobilization using CellLux™ Fluorescence Cell Imaging technology. The aim of this work was to use primary cultured rat astrocytes and astrocytic glial cells to characterize the allosteric modulators of mGluRs by measuring intracellular Ca2+ mobilization using CellLux™ Fluorescence Cell Imaging technology.

**MATERIALS AND METHODS**

- **Astrocyte isolation, culture and mGluR solution**
  - Ten rats were sacrificed between 24 to 48 h after birth. Rat brain were dissected into pieces with a razor blade. Isolated astrocytes were cultured in medium containing 20% FCS, 2 mM glutamine and ASA 0.5 mM in 1% Pen-Strep, 10,000 cells/well on poly-D-lysine coated 96-well clear bottom black plate (Immulon-2, Thermo Scientific). Medium was changed at day 1 and 6 after glial cell isolation. After 7 days, astrocyte cells were cultured on poly-D-lysine coated 96-well clear bottom black plate

- **Calcium mobilization measurement**
  - Brain slices were dissected into pieces with a razor blade. Isolated astrocytes were cultured in medium containing 20% FCS, 2 mM glutamine and ASA 0.5 mM in 1% Pen-Strep, 10,000 cells/well on poly-D-lysine coated 96-well clear bottom black plate. Medium was changed at day 1 and 6 after glial cell isolation. After 7 days, astrocyte cells were cultured on poly-D-lysine coated 96-well clear bottom black plate. Medium was changed at day 1 and 6 after glial cell isolation. After 7 days, astrocyte cells were cultured on poly-D-lysine coated 96-well clear bottom black plate.

**RESPONSE OF RAT ASTROCYTES TO DIFFERENT LIGANDS INDUCED CALCIUM MOBILIZATION**

- **Concentration-response (CIV) curve of quisqualate and L-glutamate on calcium mobilization**
  - L-glutamate and quisqualate as index the mGluRs inducing the transient calcium increase in rat astrocytes. The potency of Glutamate-metabotropic receptors is one of the most abundant and best-characterized metabotropic glutamate receptors in astrocytic glial cells (4). The stimulation of mGluR5 in the allosteric regions of the receptor (2, 3).

- **Effect of ionotropic glutamate receptor antagonists CNQX and L-AP5 on L-glutamate induced calcium mobilization**
  - L-glutamate and quisqualate in astrocytes promote Ca2+ oscillation, protein kinase C activation and induces signal transduction cascades, making astrocytes good in pharmacological tools in discovery and development of drugs active at mGluRs (5).

- **Effect of CDPPB on concentration-reponse curve of quisqualate induced calcium mobilization**
  - CDPPB 10 µM induced a parallel leftward shift of the concentration-response curve. Using the Gaddum equation pKB = log (EC 50 glutamate/EC 50 quisqualate), pKB = 2.6 folds. The negative allosteric modulator CDPPB had no agonist activity but antagonizes totally the response to an EC20 concentration of quisqualate.

- **Effect of L-AP5 on calcium mobilization**
  - L-AP5 (20 µM) completely blocked the quisqualate-evoked calcium transient.

- **Concentration-response curve of positive allosteric modulator MPEP on L-glutamate induced calcium mobilization**
  - MPEP caused slight right shift of the glutamate concentration-response curve with the decreased maximal calcium transient. Using the Gaddum equation pKB = log (EC 50 glutamate/EC 50 quisqualate), pKB = 2.6 folds.

- **Concentration-response curve of positive allosteric modulator DPPB on L-glutamate induced calcium mobilization**
  - DPPB had no agonist activity but antagonizes totally the response to an EC20 concentration of quisqualate.

**REFERENCES**