

Activation, potentiation and inhibition of AMPA receptors on the Patchliner

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Summary

AMPA receptors are cation-permeable ionotropic glutamate receptors of the non-NMDA receptor sub-family¹. To date four subunits, GluA1-4, have been identified¹⁻³ which are of similar size (approx. 900 kDa) and share 68-73% amino acid sequence identity³. The human GluA2 subunit is encoded by the GRIA2 gene located on the 4q32-33 chromosome². Each of the 4 subunits has four distinct domains: an extracellular amino acid terminal domain (ATD); the extracellular ligand binding domain (LBD); the transmembrane domain (TMD) with 3 transmembrane segments (M1, M3 and M4) and 1 cytoplasmic facing re-entrant loop (M2); and an intracellular carboxy-terminal domain^{1,2}. The functional receptor exists as a tetramer, either as homomers or heteromers (GluA1 and GluA4)¹. The vast majority of excitatory fast synaptic transmission in the mammalian central nervous system is mediated by AMPA receptors of differing subunit combinations¹. It is well known that glutamate is a neurotoxin and it is proposed that overactivation of ionotropic glutamate receptors may underlie many neurodegenerative disorders such as ischemic stroke, epilepsy, Parkinson's and dementia, amongst others². Enhancement of AMPA receptor activation by, for example, BDNF, has been proposed to have beneficial effects on learning and memory and has potential therapeutic value in the treatment of depression, Huntington's and Parkinson's diseases¹.

Here we present data collected on an 8-channel Patchliner showing recordings of GluA2-mediated currents. Glutamate activated GluA2 receptors with an EC_{50} similar to those

reported in the literature^{4,5}. CNQX inhibited and LY404187 enhanced GluA2-mediated responses.

Results

hGluA2 expressed in HEK293 cells was activated using increasing concentrations of glutamate (Figure 1). hGluA2 started to activate at a glutamate concentration of 10 μ M. At the highest concentration tested (3 mM) a rise time of 12.5 ms was estimated (Figure 1, inset). The EC_{50} for glutamate was calculated to be $36.1 \pm 6.4 \mu$ M ($n = 4$) in good agreement with the range found in the literature for GluA2^{4,5}.

The application speed of glutamate (100 μ M) had a large influence on activation and deactivation kinetics (Fig. 1C), at a slower speed of application, the initial fast peak was not observed. NB: Multi-hole chips (4 holes per well) were used.

hGluA2 expressed in HEK293 cells was reproducibly activated (Figure 2A) using repetitive (at least 4 x) applications of a glutamate concentration close to the EC_{50} (30 μ M).

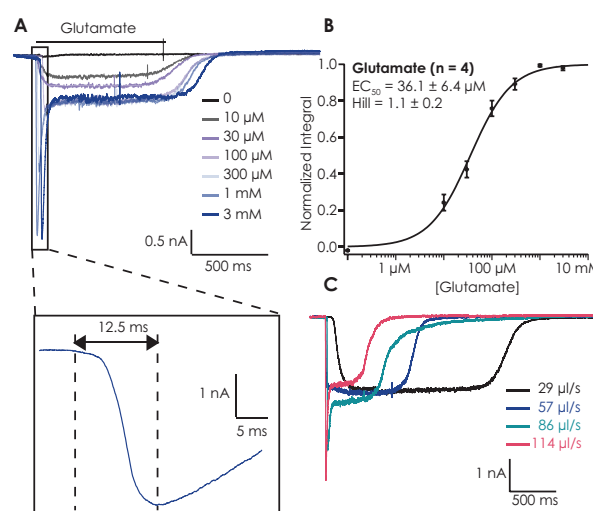


Figure 1: **A** Example traces showing activation of GluA2 by Glutamate. A rise time of 12.5 ms was calculated at 3 mM (inset). **B** Concentration response curve for an average of $n = 4$ wells. **C** Application speed greatly affects activation and deactivation kinetics of GluA2.

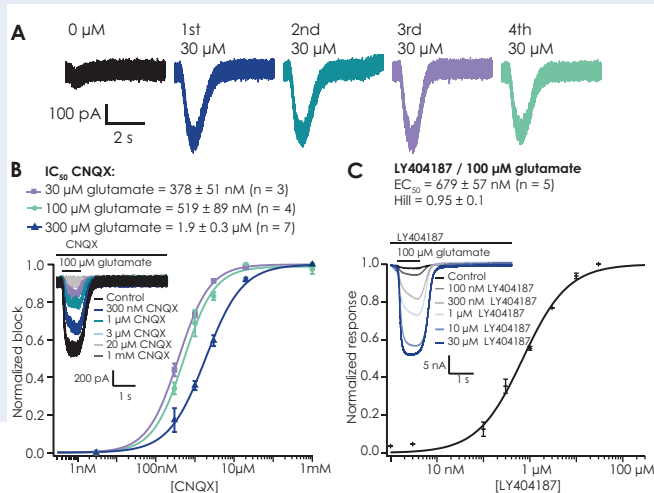


Figure 2: A GluA2 could be repetitively activated at least 4 times in the cell with similar peak amplitudes (currents shown on the same scale). **B** GluA2 was inhibited by CNQX, the IC_{50} was dependent on glutamate concentration. **C** GluA2 was potentiated by LY404187 of GluA2 responses in a concentration-dependent manner.

The GluA2 response was blocked by CNQX in a concentration dependent manner with an IC_{50} within the range found in the literature for GluA2-containing receptors⁶. The potency of block was dependent on glutamate concentration (Figure 3A). The positive allosteric modulator (PAM) LY404187 potentiated the glutamate response (100 μM glutamate) with an EC_{50} of 679 ± 57 nM (n = 5) in good agreement with the literature for GluA2⁷. The potency was not affected by glutamate concentration (data not shown).

In conclusion, fast exchange of the external solution on the Patchliner can be used to reliably activate GluA2 receptors. Studies were undertaken to investigate modulators of GluA2 receptors, including inhibitors or PAMs. In this way, new compounds with potential for treating, for example, epilepsy, Parkinson's and Huntington's diseases can be identified.

References

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Methods

Cells

HEK cells expressing hGluA2 were kindly provided by SB Drug Discovery.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard cell culture protocol. Cells were induced to express hGluA2 by incubation in 1 $\mu\text{g}/\text{ml}$ tetracycline 18 - 24 hours prior to experiments. Cells were resuspended in external recording solution and stored in the CellHotel of the Patchliner before being dispensed into each well of the NPC-16 chip. Internal and external solution compositions are available upon request. Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner. Cells were held at a holding potential of -80 mV. To achieve short exposure times, solutions were stacked in the robotic pipettor. First, wash solution was aspirated followed by 40 μl of the agonist-containing solution and then applied to the cell at a speed of 57 $\mu\text{l}/\text{s}$ (or at the other speeds indicated). Cells were pre-incubated in CNQX or LY404187 prior to co-application with glutamate.

