Expression and pharmacology of GluA2-containing AMPA receptors in cell lines and stem cell-derived neurons

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Introduction

The vast majority of excitatory neurotransmission is mediated by AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors[1]. The functional receptor exists as a tetramer, either homomers or heteromers, from a repertoire of 4 different subunits, GluA1 – GluA4[1]. 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[2]. Enhancement of AMPA receptor activation by, for example, BDNF, has been proposed to have beneficial effects of learning and memory and has potential therapeutic value in the treatment of depression, Huntington's and Parkinson's diseases[1]. We have used GluA2 receptors expressed in HEK cells on 3 different automated patch clamp systems recording from either 1, 8 or 384 wells simultaneously. GluA2 was activated by glutamate, inhibited by CNQX and potentiated by LY404187.

In addition to GluA2 expressed in HEK cells, we also recorded glutamatergic-enriched cortical neurons derived from induced pluripotent stem cells on an automated patch clamp platform. In these neurons we recorded various voltage-gated channels. In addition, glutamate responses were recorded which were potentiated by LY404187.

Activation of GluA2 expressed in HEK cells

Port-a-Patch

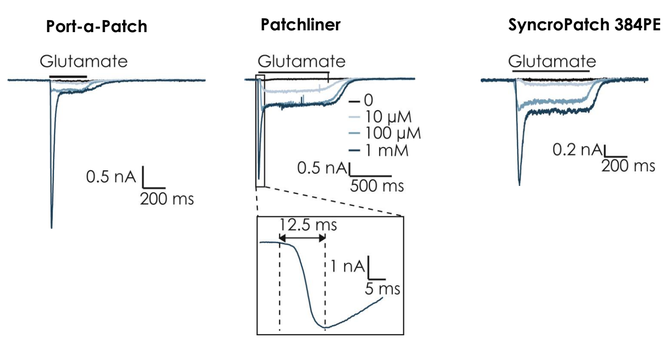
Glutamate

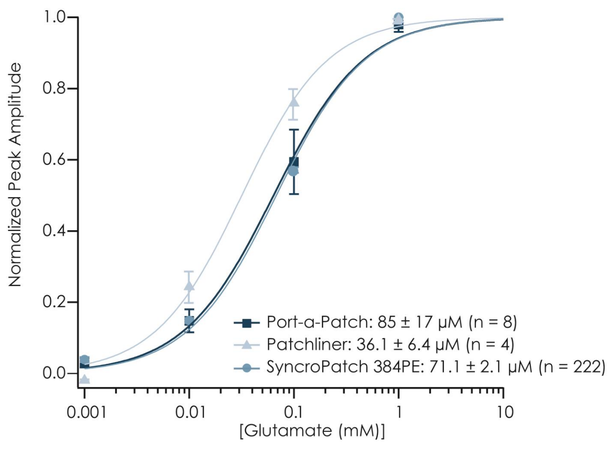
Patchliner

Glutamate

SyncroPatch 384PE

Glutamate



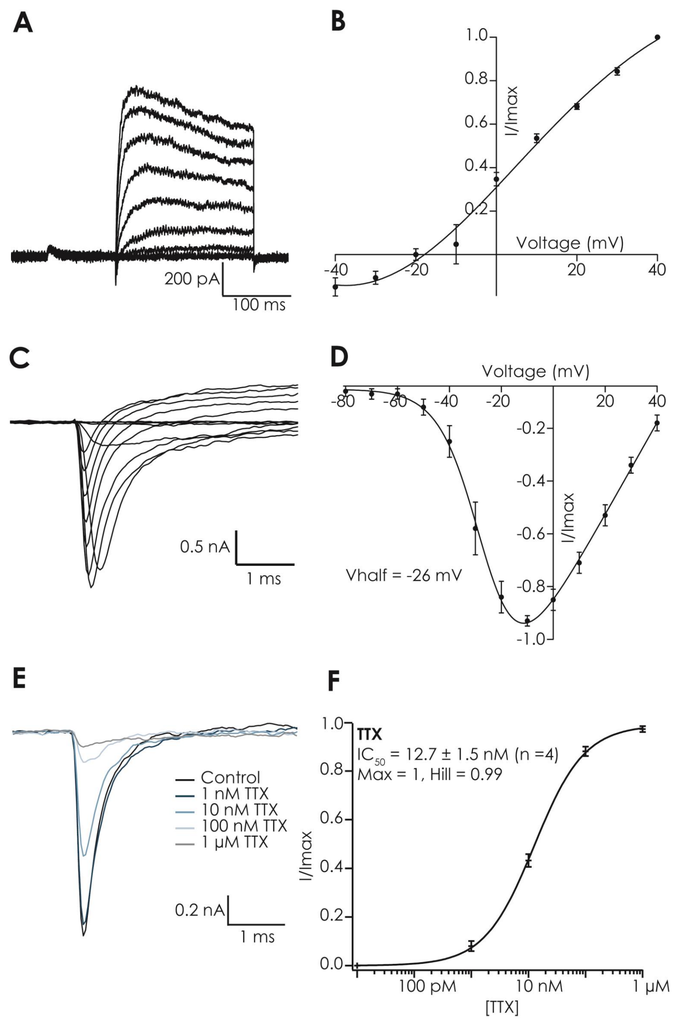


GluA2 expressed in HEK cells was recorded on the Port-a-Patch (left), Patchliner (middle) and SyncroPatch 384PE (right) where 1, 8 or 384 wells are recorded in parallel, respectively.

GluA2 was activated by glutamate in a concentration-dependent manner with EC_{50} similar across all 3 platforms and in agreement with the range found in the literature[3,4] (lower panel). Cumulative concentration response curves were performed on each cell. The data was normalized to the highest concentration (1 mM) and the concentration response curves for all 3 platforms is shown here overlaid.

Cells kindly provided by SB Drug Discovery.

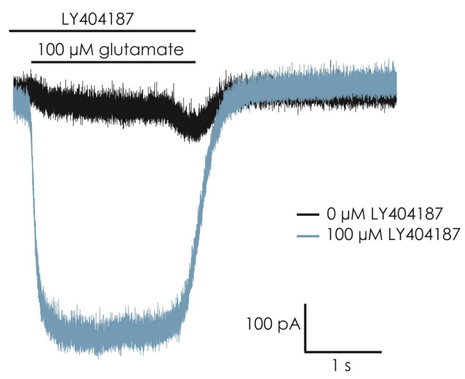
Voltage-gated ion channels expressed in hiPSC-derived neurons



iCell® GlutaNeurons were used on the Patchliner. Voltage-gated potassium currents were recorded (**A**) using a 100 ms pre-pulse to -40 mV followed by 200 ms voltage step increasing in 10 mV increments to +40 mV. The average IV plot for 6 cells is shown (**B**). Voltage-gated Na^+ currents were also recorded from the iCell® GlutaNeurons (**C**). The average IV plot for 12 cells is shown in (**D**). A Boltzmann equation revealed a V_{half} of -26 mV. The Na_V was blocked by TTX in the nM range (**E, F**), IC_{50} = 12.7 ± 1.5 nM (n = 4) indicating a TTX-sensitive channel. The IC_{50} for TTX agrees well with the IC_{50} obtained for iCell® Neurons[7].

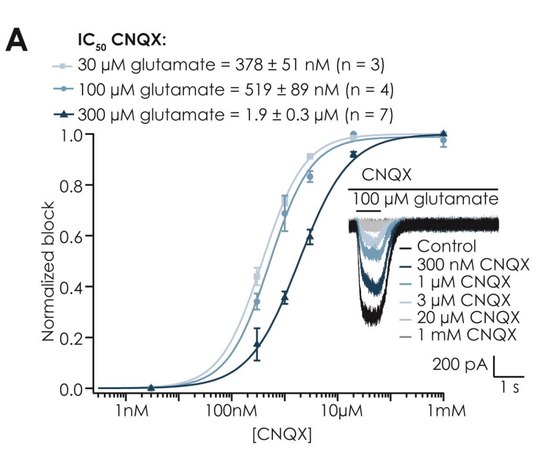
Cells kindly provided by Cellular Dynamics International.

Activation of glutamate channels in iCell GlutaNeurons



The presence of glutamate receptors was investigated in iCell® GlutaNeurons. Although only a minimal current could be observed upon application of glutamate, the response was enhanced by the AMPA-specific PAM, LY404187. This indicates the presence of AMPA receptors in these neurons. The presence of other ligand-gated ion channels, e.g. NMDA was not yet fully investigated.

Inhibition and potentiation of GluA2 expressed in HEK cells



GluA2 expressed in HEK cells was activated by glutamate and inhibited by CNQX on the Patchliner in a concentration-dependent manner. Interestingly, the IC_{50} of CNQX was dependent on the glutamate concentration (**A**). A similar IC_{50} value was also obtained using the SyncroPatch 384PE (588 nM, n = 128) using 100 µM glutamate, in good agreement with the literature[5].

The positive allosteric modulator (PAM), LY404187, was pre-incubated and co-applied with 100 µM glutamate. LY404187 enhanced the glutamate response with an EC_{50} of 679 ± 57 nM (n = 5) on the Patchliner (**B**) and 443 nM (n = 367) on the SyncroPatch 384PE (not shown), in good agreement with the literature[6].

Conclusions

- GluA2-mediated responses expressed in HEK cells could be reliably recorded on the Port-a-Patch, Patchliner and SyncroPatch384PE with similar EC_{50} values for glutamate.
- GluA2 expressed in HEK cells was blocked by CNQX and the IC_{50} was dependent on glutamate concentration.
- GluA2 expressed in HEK cells was potentiated by the AMPA-specific PAM, LY404187.
- Voltage-gated Na^+ and K^+ currents were recorded in glutamatergic cortical neurons derived from iPSCs, iCell® GlutaNeurons
- AMPA receptors are expressed in iCell® GlutaNeurons (CDI), a response to glutamate was measured in the presence of LY404187.

References

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