NMDA Receptor Modulators in QPatch

Introduction

N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors permeable to Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\). To be activated, they need to bind to glutamate (via GluN2 subunits), glycine (via GluN1) and release the Mg\(^{2+}\) blockade by membrane depolarization. The majority of NMDARs are tetrameric complexes, consisting of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. GluN2 is coded by a single gene with at least eight different splice variants; four different GluN2 genes originate GluN2A, GluN2B, GluN2C, and GluN2D subunits. NMDARs containing different GluN2 subunits have different pharmacological and kinetic properties.

NMDA receptor modulators can be studied using a variety of techniques. Many of them are available in Evotec, including those listed here below:

- NMDAR Stable Cell Lines
- GluN1-GluN2B Current Stability in QPatch
- Glutamate Deactivation Kinetic Studies in QPatch: CIQ on GluN1-GluN2B Receptor
- Onset/offset Kinetic Studies in QPatch: Ketamine on GluN1-GluN2B Receptor

Objective

The objective of this poster is to illustrate few methodologies which can be applied to characterize various classes of NMDA receptor modulators in recombinant cell lines, using QPatch automated system.

NMDAR Stable Cell Lines

CHO cell lines stably expressing dithioether NMDARs [hGluN1-hGluN2A, hGluN1-hGluN2B, hGluN1-hGluN2C, or hGluN1-hGluN2D] were generated in Evotec. Refseq protein accession numbers of NMDAR subunits are as indicated below:

- hGluN1: NP_015966
- hGluN2A: NP_000824
- hGluN2B: NP_000825
- hGluN2C: NP_000826
- hGluN2D: NP_000827

QPatch Protocol for Inward or Outward Current Measurement

- Inward current measurement: extracellular solution did not contain magnesium
- Fixed recording voltage at holding potential: -50 mV
- Outward current measurement: extracellular solution contained 1 mM MgCl\(_2\)
- Cells were stimulated from a holding potential of -80 mV to +60 mV with a 2 s step pulse, followed by a 2 s ramp to -80 mV, which allows visualization of Mg\(^{2+}\) block
- 100 µM glutamate plus 10 µM glycine were added during 600 µs, after 500 ms from the deactivation (as indicated by pipette drawing)
- Steady state current amplitude could be measured at the end of the 600 µs pulse

GluN1-GluN2B Current Stability in QPatch in Outward Current Protocol

- Saline or agonists (e.g., 10 µM glutamate + 1 µM glycine) were added every 40 seconds, by QPatch protocol for outward current measurement using CHO cells expressing hGluN1-hGluN2B
- Agonists elicited current decreased down to an average 78% (n=5) of its original value, 400 seconds after agonists first application

Glutamate Deactivation Kinetic Studies in QPatch: CIQ on GluN1-GluN2B Receptor

- Liquid Peristaltic:
  - 10 µM CIQ: 9 µl CIQ
  - 20 µl saline

Onset/offset Kinetic Studies in QPatch: Ketamine on GluN1-GluN2B Receptor

- Liquid Peristaltic:
  - 10 µM ketamine

Conclusions

- CHO cell lines expressing human NMDA receptors composed of GluN1-1a subunit in combination with GluN2A, GluN2B, GluN2C, or GluN2D, have been generated
- Cell lines were profiled in automated QPatch. Excellent match of agonists, antagonists and modulators potencies is observed between manual patch clamp and automated QPatch data.
- On- and off-set kinetic studies for NMDAR modulators were performed in QPatch. A good match of data was obtained with manual patch clamp data (not shown), for fast compounds such as ketamine
- Glutamate deactivation kinetic studies for NMDAR modulators were performed in QPatch. A good match of data was obtained with manual patch clamp data (not shown), for fast compounds such as ketamine
- The four cell lines constitute an useful tool to profile compounds acting at various isoform of NMDARs

**Legend:**
- L.: hNMDAR stable cell lines
- T: hNMDAR transiently transfected cells
- N: rat primary neurons
- S: rodent brain slices
- H: rat brain homogenates

**Manual patch clamp Current / potential 1**
- L. / T. / N. / S.

**IonWorks/SyncroPatch Current 384**
- L.

**FLIPR Calcium 384**
- L. / N.

**QPatch Protocol for Inward or Outward Current Measurement**
- Current / potential 1
  - L. / T. / N. / S.

**Scientific Skills Master Potential**
- 4 S.

**3Brain CMS HD MEA Potential**
- 4.096 S.

**Envision pERK**
- 384 N.

**Brandon filtration unit**
- [H]-MK-801 binding 96 H.

**S: rodent brain slices; H: rat brain homogenates.**