Evaluation of positive allosteric modulators of SK2 channels using QPatch

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Introduction

Small-conductance Ca^{2+}-activated K^+ (SK) channels mediate after-hyperpolarization in neurons and dampen the firing frequency of action potentials. Calmodulin is constitutively associated with the SK2 channels and serves as the Ca^{2+} sensor for the SK-calmodulin complex. The SK2 channel subtype plays a key role in the regulation of excitability of Purkinje cells in the cerebellum. Given their importance in Purkinje cells, SK2 channels are a promising drug target for ataxia, a movement disorder.

Automated patch clamp (APC) machines, such as QPatch, have been used in pharmaceutical industry to study drug interaction with varieties of ion channels. Over the past decade, the technology has contributed significantly to pharmaceutical research and drug discovery. Here, we report to use QPatch as a tool for testing and evaluating the positive allosteric modulators of SK2 channels. A stable cell line of the rat SK2 channel tagged with GFP was established through transfection of HEK293 cells followed by puromycin selection and enrichment using repeated GFP fluorescence-activated cell sorting.

Positive allosteric modulators were tested under whole-cell voltage clamp configuration with automated QPatch. The compounds that positively modulate the SK2 channels in the automated QPatch was further tested with the inside-out patch manual recordings. The results from automated whole-cell recordings and inside-out patch manual recordings were consistent.

Data collection and analysis

QPatch

Trypsinized SK2 HEK293 cells were resuspended in serum free medium at 3-5x10^6 cells/ml for APC experiments. Voltage protocols were applied as follows: Vh=-90 mV, ramp from -120 mV to +40 mV, 350 ms. Application protocol was performed sequentially as following: saline (as control); testing compound (different concentrations were studied); NS309 10 µM (as a positive control for normalization); Apamin 300nM (as a negative control). Drug effects were analyzed as concentration-dependent response (Hill fit and EC_{50}) using Sophion Analyzer 6.5 and (Graphpad Prism8.0).

Manual inside-out patch

SK currents were recorded using an inside-out patch configuration, with an Axon200B amplifier (Molecular Devices) at room temperature. Currents were recorded by repetitive 1-s-voltage ramps from – 100 mV to + 100 mV from a holding potential of 0 mV. To construct the concentration-dependent potentiation of channel activities by compounds, the current amplitudes at – 90 mV in response to various concentrations of compounds were normalized to that obtained at maximal concentration of compounds. The normalized currents were plotted as a function of the concentrations of compounds. EC_{50} values and Hill coefficients were determined by fitting the data points to a standard concentration–response curve (γ = 100/(1 + (X/EC_{50})^ – Hill)).

Conclusion

1. QPatch, an automated patch clamp (APC) machine, can be used to study drug interactions with SK channels.
2. NS309 potentiates SK2 current with a potency of 1.11 µM in the QPatch study. Comparable result was also obtained from manual inside-out patch clamp study.

Materials and methods

Cells and compounds

SK2 currents were recorded on the QPatch automated patch clamp (APC) platform. NS309, the positive modulator for SK2 channels, was purchased from Sigma-Aldrich.

Solutions

QPatch

Extracellular solution (in mM): NaCl 145, $CaCl_2$ 2, $MgCl_2$ 1, KCl 4, HEPES 10, and Glucose 10, pH=7.4 (NaOH), ~305mOsm.

Intracellular solution (in mM): KCl 120, $CaCl_2$ 5.4, $MgCl_2$ 175, KOH/EGTA 31.25/10, HEPES 10, and Na2-ATP 4, pH=7.2 (NaOH), ~294-301 mOsm.

Manual inside-out patch

Extracellular solution (in mM): 140 KCl, 10 Hepes (pH 7.4), 1 $MgSO_4$. The intracellular solution (in mM): 140 KCl, 10 Hepes (pH 7.2), 1 EGTA, 0.1 Dibromo-BAPTA, and 1 HEDTA was mixed with Ca^{2+} to obtain the desired free Ca^{2+} concentrations, calculated using the software (https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcS.htm)

Fig. 1: QPatch II, the second generation of QPatch that carries out automated whole-cell voltage clamp recordings.

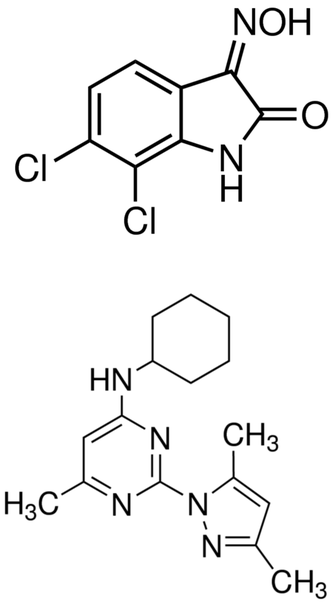

Fig. 2: Structures of NS309 & CyPPA, two SK2 potentiators studied in this poster.

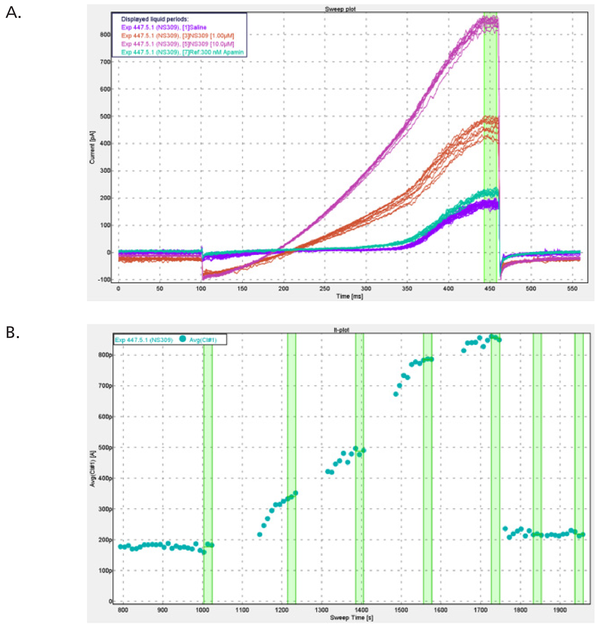

Fig. 3: Concentration-response for NS309 on SK2 current. **3A:** Raw current traces of SK2 currents at 0, 1, 10 µM NS309, and 300 nM Apamin. V-protocol shown in inset. **3B:** I-t plot correspond to the current traces.

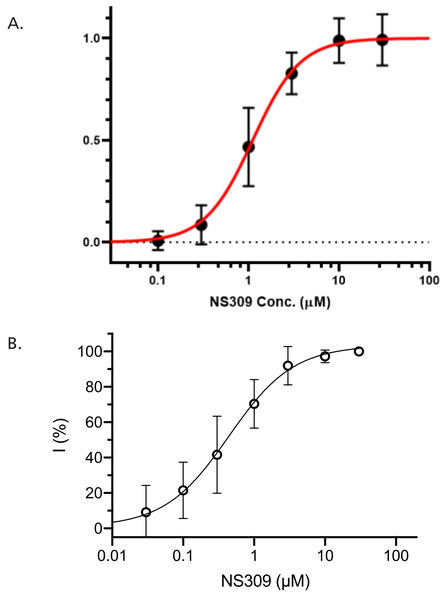

Fig. 4: Concentration response curve (CRC) for NS309. **4A**: CRC for NS309 obtained from QPatch. Data represent mean +SD. Number of cells for each concentration varied from 17 to 49 and the EC_{50} is estimated 1.11 µM. **4B**: CRC for NS309 obtained from manual inside-out patch clamp recording. Data represent mean + SD. The EC_{50} is estimated 0.55µM