APPLICATION OF QPATCH 16 FOR DRUG SCREENING OF LIGAND-GATED ION CHANNELS

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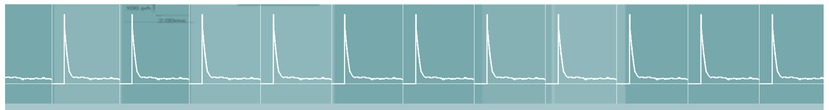


The QPatch 16 screening station is a second generation automated patch-clamp system based on planar silicon chip technology. QPatch 16 has previously been employeed for a series of screening studies on a number of voltage-gated ion channels (e.g. hERG, KCNQ4, $Na_v1.2$, $Na_v1.4$ and $Na_v1.5$).

Recently, ligand-gated ion channels (LGIC) including $GABA_A$, nAChR and ASIC have been targeted with QPatch 16. We here report a study in which the $GABA_A$ receptor ($\alpha_1\ \beta_2\ \gamma_2$) was targeted with 4-5 concentrations of an agonist (GABA), an antagonist (bicuculline) and a modulator (chlordizepoxide). Subsequently we report a study on acid sensitive ion channels (ASIC1a) in which the effect of pH, i.e. protons which serve as the ligand, was examined.

THE QPatch SCREENING STATION

In QPatch 16 patch-clamping takes place on the disposable QPlate which contains 16 individual patch-clamp positions that are operated in parallel. Salines and compounds (agonists, antagonists or modulators) are applied by four pipette heads that afford more efficient assays and faster throughput for ion channel drug discovery. Cultured cells expressing the target ion channel are kept in culture medium in an on-board stirred reservoir for up to four hours. Prior to testing the cells are transferred to an on-board mini centrifuge where they are spun down and resuspended in Ringer's solution (washed) twice before being applied to the pipetting wells in the QPlate. Gigaseals are formed upon execution of a combined suction/voltage protocol. Further suction leads to whole-cell configuration. Solutions and compounds are applied through integrated glass-coated flow channels. Liquid flow is laminar with exchange time constants in the range 50-100 ms. After application all fluids are collected in the built-in waste reservoir (100 µL).

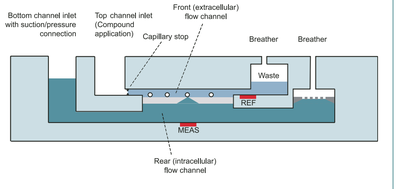
RINGERS

Extracellular Ringer's solution consisted of (in mM): 145 Na^+, 4 K^+, 2 Ca^{2+}, 2 Mg^{2+}, 155 Cl^-, 10 HEPES (pH 7.4). Intracellular Ringer's solution consisted of (in mM): 120 K^+, 1.8 $Mg2^+$, 123.6 Cl^-, 10 EGTA, 10 HEPES (pH 7.2).

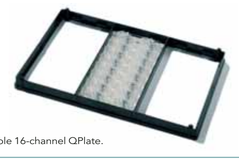
DATA ANALYSIS

Recorded ion channel whole-cell currents were stored in an integrated database (Oracle) along with data on suction pressure, seal resistance, series resistance and capacitances (C_{slow} and C_{fast}). Drug effects were analysed as function of time (I-t plot) and concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software.

The QPatch™ 16 automated patch-clamp station.

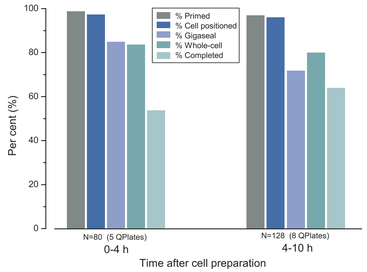


Transection of chip assembly illustrating front and rear flow channels, capillary stop, and reference and measuring electrode (REF and MEAS). Both electrodes connect to bottom site for interfacing to QPatch amplifier.

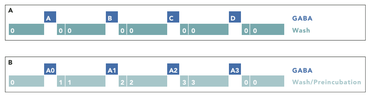


The disposable 16-channel QPlate.

SUCCESS RATES AND CELL STABILITY



QPatch success rates for priming, cell positioning, gigaseal formation, whole-cell configuration and completion of protocol (CHO-hERG study). First group of bars represent data from 0-4 hours after cell preparation. Second group of bars represent data from 4-10 hours after cell preparation.

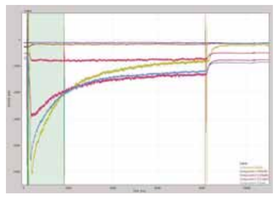


COMPOUND APPLICATION PROTOCOLS:

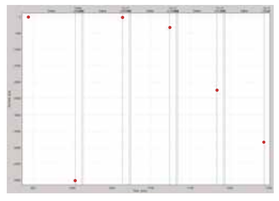
A **Agonist protocol:** A sequence of exposures to extracellular solutions indicated. Green boxes labelled 0 indicate pure Ringer's solution. Blue boxes labelled A-D indicate increasing concentrations (400 nM-50 µM) of the agonist (γ-amino-butyric acid, GABA) added for 5 seconds.

B. **Antagonist protocol:** Sequence of extracellular solutions indicated. Olive boxes labelled 0-3 indicate pure Ringer's solution (0) or Ringer's solutions added increasing antagonist concentrations (1-3) for 60 seconds each. Blue boxes indicate 2-second exposures to Ringer's solutions containing the agonist (GABA) at a concentration of 10 µM.
Modulator protocol: identical to antagonist protocol, except that only one concentration (20 µM) of the modulator was employed.

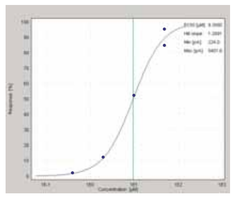
$GABA_A$ - AGONIST STUDY



Recordings of whole-cell $GABA_A$ currents elicited in response to pure Ringer's solution and to 5 increasing concentrations of GABA. The current rise-time at maximal GABA concentration (50 µM) was 60 msec.

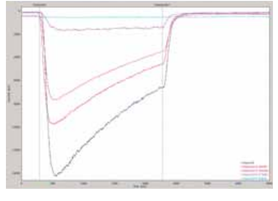


I-t plot of maximal current amplitudes recorded at each GABA concentration.

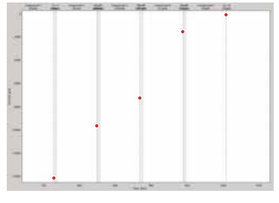


Concentration-response relationship. EC_{50} = 9.4 µM. Hill slope = 1.3.

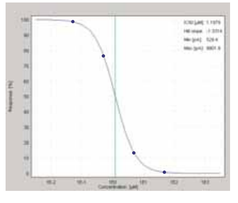
$GABA_A$ - ANTAGONIST STUDY



Recordings of whole-cell $GABA_A$ currents elicited in response to 10 µM GABA in the presence of either pure Ringer's solution or Ringer's solution added 4 increasing concentrations of the antagonist bicuculline.

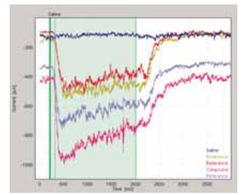


I-t plot of maximal current amplitudes recorded at each bicuculline concentration.

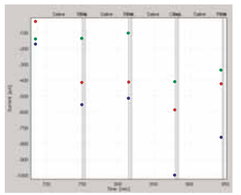


Concentration-response relationship. EC_{50} = 1.2 µM. Hill slope = -1.3

$GABA_A$ - MODULATOR STUDY

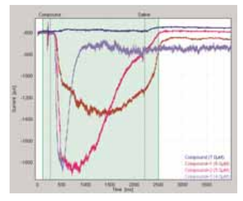


Recordings of whole-cell $GABA_A$ currents elicited in response to 10 µM GABA in the presence of either pure Ringer's solution (Reference, red, blue and yellow traces) or Ringer's solution added 20 µM chlordizepoxide (Compound, purple trace). The current rise-time was 80 msec in the presence of chlordizepoxide.

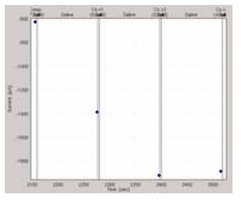


I-t plot of maximal current amplitudes recorded at each bicuculline concentration. Blue: raw current; green: leak current; red: leak-subtracted current.

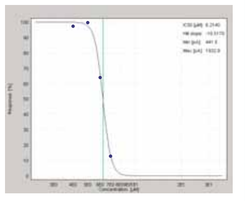
ASIC1a - AGONIST STUDY



Recordings of whole-cell ASIC1a currents elicited in response to 4 increasing extracellular proton concentrations (pH 7, 6, 5, 4).



I-t plot of maximal current amplitudes recorded at each pH.



Concentration-response relationship. Half-maximal current was obtained at pH 6.2 (proton concentration 602 nM).

CONCLUSION

Compound screening on ligand-gated ion channels ($GABA_A$ and ASIC1a) can be performed efficiently with the QPatch 16 automated patch-clamp system in order to characterize the effects of agonist, antagonist and modulators. In comparison to other systems on the market, the QPatch's four pipette heads afford more efficient assays and higher throughput for gigaseal quality patch clamping.
The analyses presented here comprise I-t and concentration-response relationships, and rise-time determinations. The EC_{50} and IC_{50} values determined for the concentration-response relations in the present study are comparable to values listed in the literature (cf. e.g. Boileau et al., Neuropharmacol., 43:659, 2002).