CHARACTERIZATION OF hERG BLOCKERS USING THE AUTOMATED QPatch-16 SCREENING SYSTEM

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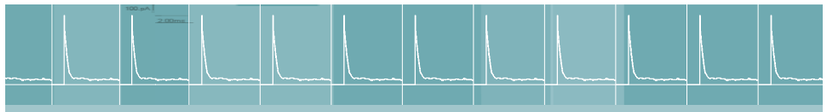


The QPatch™ technology has been developed to significantly increase throughput in ion channel drug screening. It is based on planar glass-coated silicon chips with micro-etched patch-clamp holes. Extra- and intracellular Ringer solutions are applied by miniature flow channels, which ensures laminar flow and short fluid exchange times (<100 ms). The walls of the flow channels are covered with non-polymer materials (glass and silicon) to minimize problems with non-specific binding of 'sticky' compounds.

Below is shown the QPatch-16, the first instrument based on the QPatch technology. It runs 16 parallel whole-cell patch-clamp experiments simultaneously with success rates of 50-80 %. Cells are maintained in growth medium in an onboard cell storage facility for up to 4 hours until shortly before an experiment. At that time they are automatically spun down in a miniature centrifuge, washed, and resuspended in Ringer's solution before being applied to the patch-clamp site.

We have determined the IC_{50} values for 32 hERG channel blockers in a blind test and made a comparison with IC_{50} values obtained in conventional manual patch-clamp.

MATERIALS AND METHODS

CELL CULTURE

The ion channels were expressed in CHO cells expressing hERG. This cell line was grown according to standard culturing protocols. After harvest cells were kept for up to 4 hours in the QPatch cell storage facility on the platform with no significant change in quality or ability to patch.

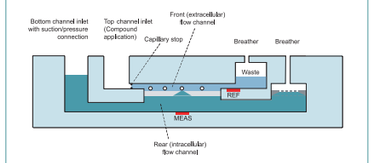
ELECTROPHYSIOLOGY

Ion channel drug screenings were performed on QPatch-16 using. The QPlate™ which contains the 16 patch-clamp sites, each constituting a complete patch-clamp unit with silicon chip, flow channels, electrodes, and waste reservoir (Kutchinsky et al., 2003). The QPlate is in a standard microtitre plate (MTP) format. The patch-clamp hole (micro-etched in the chip) is approximately 1 µm in diameter and has a resistance of ~2 MΩ.

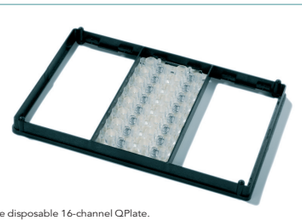
Voltage-clamp protocol: The membrane potential was clamped to a holding potential of -60 mV, then depolarized to 20 mV for 2000 ms, before stepping to -60 mV. Outward hERG tail currents were recorded at -60 mV.

SOLUTIONS AND DRUGS

The physiological Ringer solutions consisted of (in mM):Extracellular Na^+ Ringer: 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4), 10 glucose.
Intracellular K^+ Ringer: 120 KCl, 5.4 KCl_2, 1.8 $MgCl_2$, 10 KOH/EGTA, Na_2-ATP, 10 HEPES (pH 7.2).
Unknown test compounds for the blind study were provided by Aventis Pharmaceutical (Bridgewater, NJ, USA).
Reference compounds were kindly supplied by NeuroSearch (Ballerup, Denmark) and another major pharmaceutical company. Verapamil was from Sigma, Switzerland. rBeKm-1 was from Alomone Labs, Israel.

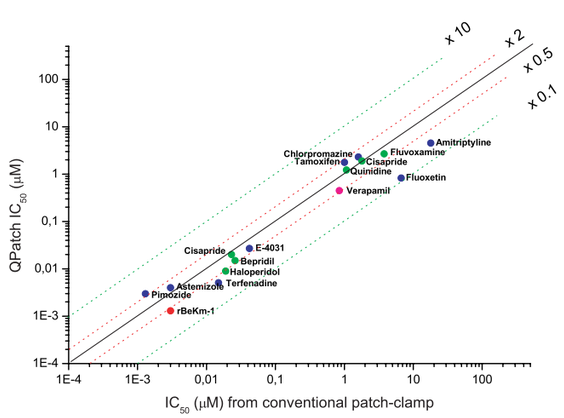


Transection of chip assembly illustrating front and rear flow channels, capillary stop, and reference and measuring electrode (REF and MEAS). Both electrodes connect to bottom site for interfacing to QPatch amplifier.



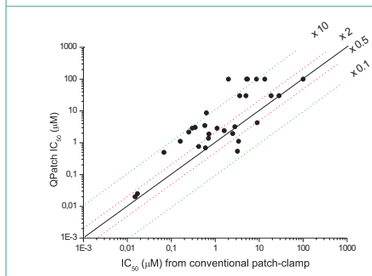
The disposable 16-channel QPlate.

The complete QPatch™ screening station.



TEST OF REFERENCE COMPOUNDS

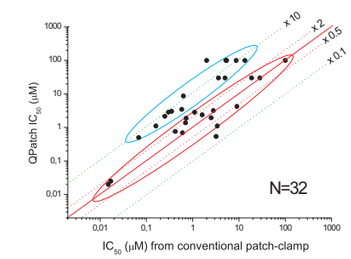
QPatch-16 was tested on a series of 16 reference compounds with IC_{50} values spanning over 5 decades of magnitude. It is seen that the IC_{50} values determined with QPatch generally were close to the values determined with conventional patch-clamp. Each data point represents the mean of 2-6 measurements. Compounds where stored in glass-coated microtitreplates prior to experiment.



BLIND TEST OF 32 hERG BLOCKING COMPOUNDS

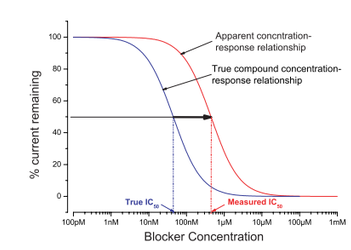
For a sample of 32 compounds IC_{50} values were determined based on data from 5 different concentrations of each compound. Compounds were prepared as stock solutions, diluted to the final test concentrations, and kept in plastic microtitre plates (MTPs) on the QPatch platform prior to use. The degree of inhibition was measured 3-6 times per concentration.

The results were subsequently compared to values obtained in manual patch-clamp studies (provided by Aventis). As a mean the QPatch IC_{50} values were a factor of 2.21 higher than the values from manual patch-clamp.



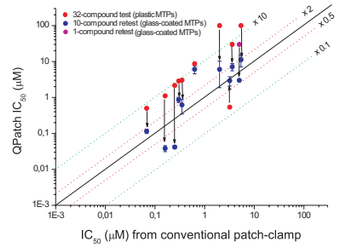
BLIND TEST OF 32 hERG BLOCKING COMPOUNDS

A closer examination of the data indicated the existence of two populations of IC_{50} values: One (indicated by a red oval) of 18 values matching the stated values within a factor of 2-5, and another population comprising 14 QPatch IC_{50} values were significantly increased relative to the stated values, as indicated by the blue oval.



NON-SPECIFIC BINDING

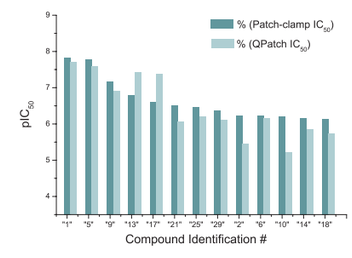
A possible explanation for the systematically increased QPatch IC_{50} values could be unspecific binding of hydrophobic ('sticky') compounds to plastic/polymer surfaces. Because the flow channels in the QPlate are made of glass and silicon, potential non-specific binding is not likely to take place here. Rather did the problem arise from pre-experimental storage of the compounds in plastic microtitre plates. Therefore, we retested 11 of the compounds that had the highest disagreement with the stated IC_{50} values in another blind study prior to which the compounds had been stored in glass-coated microtitre plates.



RETEST OF 11 COMPOUNDS

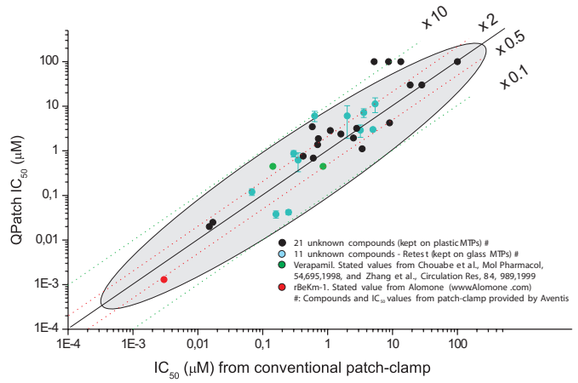
The retest of 11 compounds kept in glass-coated microtitre plates reduced the QPatch IC_{50} values in all cases except one. That one was determined to be a factor of 8 lower than the stated value in the original test, and upon retest it increased to a value not significantly different from the stated value. In two cases the QPatch IC_{50} values were reduced to values lower than the stated values. In one case the QPatch IC_{50} value did not shift significantly.

In the retest QPatch IC_{50} values were determined from 4 compound concentrations. 6 measurements were performed at each concentration. The total number of 240 measurements (data points) were done in 8 hours.



RANK OF POTENCY

The figure depicts the rank of potency as obtained upon the retest for the 13 compounds with stated IC_{50} values below 1 µM (Y-axis: -log (IC_{50})).



COMBINED RESULTS

Combination of data from the original test of the 32 compounds (kept in plastic microtitre plates) and the retest of the 11 compounds (kept in glass-coated microtitre plates). In addition is included data for verapamil and the potent hERG blocker rBeKm-1. Upon retest the QPatch IC_{50} values for the 32 compounds were as a mean a factor of 1.15 higher than the stated values. The three most deviating data points (above the grey oval) represent 3 compounds that were not included in the retest. 59% of the QPatch IC_{50} values are less than a factor of 2, and 97 % are less than a factor of 10 off the stated values.

SUMMARY

A blind test of 32 unknown hERG blockers led to QPatch IC_{50} values that were higher than the values obtained in manual patch-clamp ('stated values'). However, a retest of 11 compounds that were not allowed contact with plastic/polymer surfaces prior to the experiment reduced the discrepancy to a factor of only 1.15 off the stated values.

We conclude that there is s good agreement between IC_{50} values obtained with classical electrophysiology (patch-clamp) and with the automated QPatch technology.

The retest of 11 compounds that had not been in contact with plastic/polymer surfaces prior to the test strongly indicates that unspecific binding of 'sticky' compounds is a major problem, but that it can be avoided by storage of hydrophobic compounds in glass-coated containers. Overall, these experience demonstrate that the Qpatch technology with its glass-coated flow channels do not expose any surfaces to which sticky compounds might adhere.

REFERENCE

Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. Kutchinsky J, Friis S, Asmild M, Taboryski R, Pedersen S, Vestergaard RK, Jacobsen RB, Krzywkowski K, Schroder RL, Ljungstrom T, Helix N, Sorensen CB, Bech M, Willumsen NJ. Assay Drug Dev Technol.:1:685-93, 2003.