CFTR pharmacology determined with QPatch

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Introduction

Automated patch clamp platforms have become an indispensable tool for increasing throughput for hit detection and compound validation in drug screening. As demonstrated by Turner *et al.* 2016, QPatch is a useful tool in characterizing CFTR functionality.

Here we demonstrate how QPatch HTX can be used for evaluation of pharmacological effects. QPatch HTX is a 48 channel format automated electrophysiology system with state-of-the-art microfluidic channels system in the QPlate.

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a chloride ion channel that conducts Cl^- ions across epithelial cell membranes. The CFTR channel regulates fluid movement and is regulated by phosphorylation. The CFTR channel consists of two transmembrane domain regions and has three intracellular domains that regulate channel activity, two nucleotide binding domains and the R-domain (Regulatory domain). The gating of the CFTR channel requires both phosphorylation of PKA (cAMP-dependent protein kinase) at the unique Regulatory domain and ATP binding and hydrolysis at the Nucleotide binding site (Carson et al. 1995).

Forskolin is an activator of the CFTR channel. Forskolin works via activation of adenylate cyclase that causes ATP to generate cAMP, which in turn works on the cAMP-dependent protein kinase (PKA). PKA phosphorylates the CFTR channel and thereby opens the channel (Haws et al. 2002). Genestein is then added to keep the channel open.

Berger et al 1998, has described how fluoride (F^-) can stimulate the CFTR channel activity. Fluoride interferes through the adenylate cyclase signaling pathway, but by a different entry point than Forskolin. When PKA phosphorylates the CFTR channel with onset by F^- the channel stays open (Berger et al. 1998).

CFTR channels are constitutively open once they have been exposed to reagents activating the adenylate cyclase, which in these experiments were F^-. Therefore is was not possible to compensate for C_{fast}, C_{slow}, R_{serie} and determinate $R_{membrane}$ Instead a reference sweep recorded at the end of each experiment was subtracted all other sweeps in the same experiment.

Materials and methods

The cell line CHO-hCFTR was kindly supplied by ChanTest – part of Charles River Laboratories International, Inc.

The cells were cultured according to the Sophion standard operating procedure for CHO cells and harvested using Detachin and kept in the QStirrer on the QPatch prior to the automatic preparation.

Solutions

Intracellular saline solution:
2.7 mM $CaCl_2$, 0.88 mM $MgCl_2$, 10 mM HEPES, 60 mM KF, 70 mM KCl, 15.6 mM KOH/10 mM EGTA, 2 mM Na_2-ATP, pH = 7.2, 295 mOsm.

Extracellular saline solution:

1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 5 mM MES, 3mM KCl, 140 mM NaCl, 20 mM TEA-Cl, pH = 7.3, 290 mOsm.

Seal- and whole cell formation

After QPatch had transferred the cell suspension to the QPlate, a pressure -70 mBar was applied to obtain positioning and sealing of the cells. A whole cell protocol with pressure pulses at -150 mBar was used to obtain whole cell formation. Thereafter the membrane was clamped at -90 mV until and between the voltage protocols were executed.

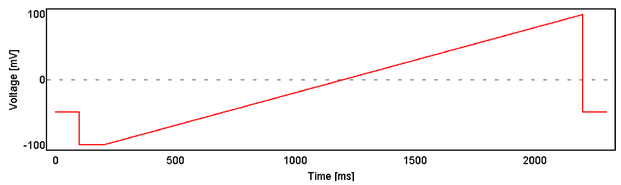
Conclusion

These experiments demonstrate that reliable recordings with the chloride conducting ion channel CFTR can be performed on QPatch using fluoride as activator of adenylate cyclase.

We have shown the ability to find the IC_{50} for $CFTR_{inh}$-172 in close correspondence to the literature values.

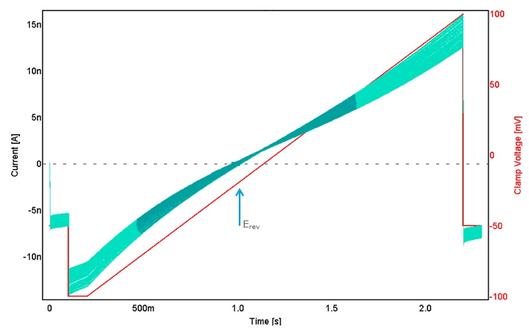
We can conclude that the overall success in obtaining stable seals and completed experiments are at a level that clearly identifies the CHO–hCFTR assay as feasible and with high quality results on QPatch.

Voltage ramp for CFTR



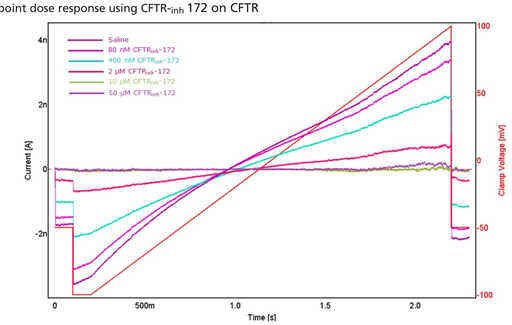
[Fig. 1]: The voltage protocol consisted of a ramp from -100 mV to +100 mV. The voltage protocol was executed every 15 seconds with 1000 Hz sampling frequency and Bessel filtering order 8.

Raw data trace



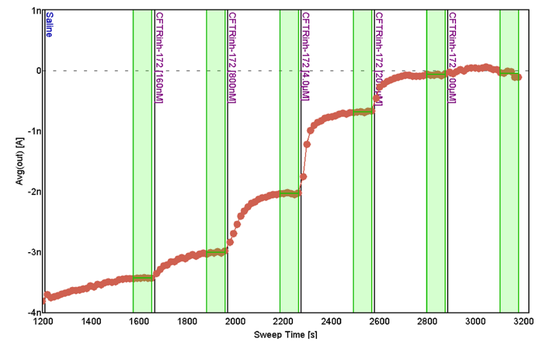
[Fig. 2]: Current traces during baseline conditions. The Blue arrow indicates E_{rev} = -20 mV

Five point dose response using $CFTR_{inh}$ 172 on CFTR



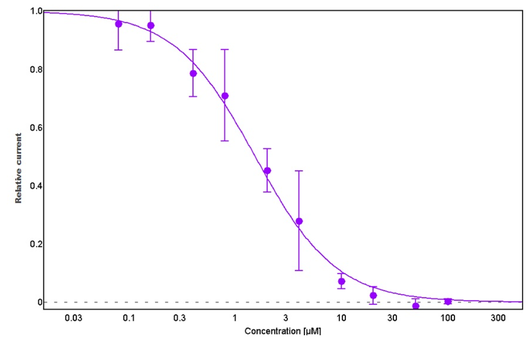
[Fig. 3]: Raw data traces in the absence as well as presence of increasing concentrations of $CFTR_{inh}$-172

Five point dose response using $CFTR_{inh}$-172 on CFTR



[Fig. 4]: Time course of the current amplitude at -100 mV and inhibition of the CFTR channel with $CFTR_{inh}$-172

Grouped Hill fit



[Fig. 5]: Hill fit for dose response with $CFTR_{inh}$-172, n=18. IC_{50} = 1.55 µM ± 0.19. Error bars are SEM

Discussion

The inward chloride current was blocked by $CFTR_{inh}$-172 with an IC_{50} = 1.55 ± 0.19 µM (n=18) Sweep subtraction has been used by which the fourth last sweep in each experiment is subtracted.

The complete block of current by the CFTR blocker $CFTR_{inh}$-172, indicates that the CHO-hCFTR cell line used in the experiments specifically overexpress the CFTR channel.

The Thiazolidinone $CFTR_{inh}$-172 is an intracellular pore blocker and is moderately permeable across membranes (Taddei *et al.* 2004). The time course illustrated in figure 4 aligns well with this site of action. In contrast to other known CFTR blockers such as Glibenclamide, the $CFTR_{inh}$-172 does not inhibit Cl^- current in a voltage sensitive manner (Ma ***et al.*** 2002). The complete mechanism underlying the block of CFTR-dependent Cl^- currents by $CFTR_{inh}$-172 is still not fully understood.

The ability of blocking the CFTR channel is very useful to treat secretory diarrhea, which are the leading cause of infant death in third world countries and a major cause of morbidity in adults. (Ma *et al.* 2002).

At concentrations fully inhibiting CFTR, $CFTR_{inh}$-172 does not prevent elevation of cellular cAMP or inhibit non-CFTR Cl^- channels, multidrug resistance protein-1 (MDR-1), ATP-sensitive K^+ channels, or a series of transporters. (Taddei *et al.* 2004)

Furthermore, the success rate in these experiments was satisfactory considering the complexity of this target.

References

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