SOPHION BIOSCIENCE

Application Report

CFTR channels activated by fluoride on QPatch

CFTR is a difficult target for most automated patch clamp platforms. Here we demonstrate reliable recordings and pharmacology of fluoride activated CFTR channels performed on the QPatch.

Summary

Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel and controls the regulation of other transport pathways. Mutations in the CFTR gene have been found to cause cystic fibrosis (CF) and congenital bilateral aplasia of the vas deferens (CBAVD).

Here we 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 determine 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.

Introduction

The CFTR channel is an important therapeutic target for treatment of Cystic Fibrosis as well as other fluid movement disorders, such as Cholera. Mutations of the CFTR gene affecting chloride ion channel function lead to dysregulation of epithelial fluid transport in the lung, pancreas and other organs. Complications from CFTR mutations include thickened mucus in the lungs with frequent respiratory infections, and pancreatic insufficiency, which can cause malnutrition and diabetes. Cystic fibrosis, is also involved in male infertility. Cystic fibrosis leads to chronic disability and reduced life expectancy. The ability to block CFTR channels are 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).

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. It 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). 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). Genistein is then added to keep the channel open.

Fluoride (F^-) can stimulate 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).

Results and discussion

CFTR channels are constitutively open once they have been exposed to reagents activating the adenylate cyclase, which in these experiments were Fluoride (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.

Both outward Cl-current and inward Cl-current was stable and E_{rev} = -20 mV (Figure 1 left). Fig 1 right shows the current traces in the absence and presence of $CFTR_{inh}$-172. The outward Cl-current was chosen for the dose response experiments with Thiazolidinone $CFTR_{inh}$-172. The time-current plot is shown in figure 2 left and the corresponding Hill fit is shown in figure 2 right.

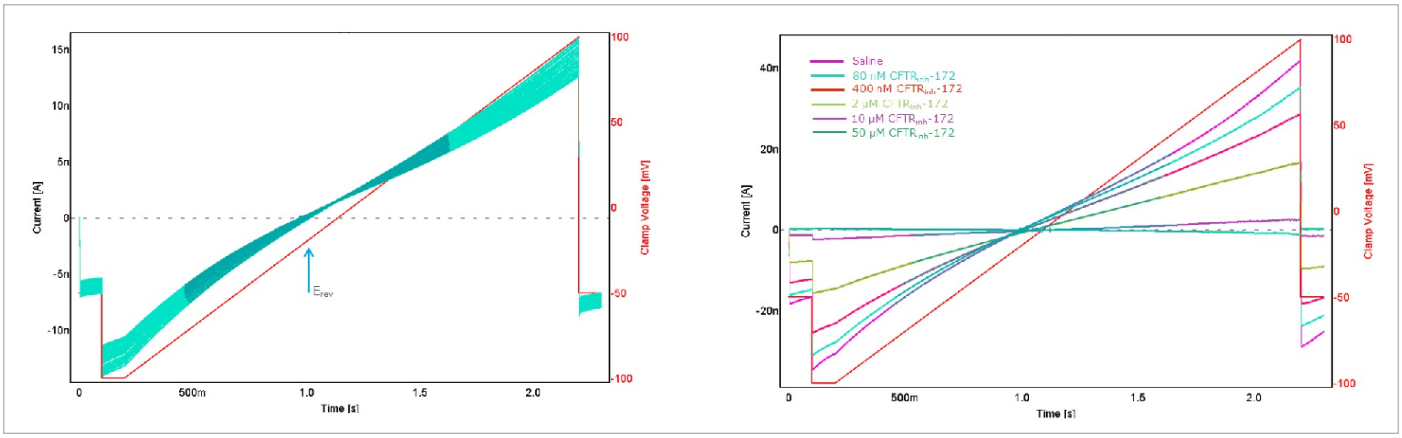

Fig. 1: Left: Current traces during baseline conditions. The Blue arrow indicates E_{rev} = -20 mV. Right: Raw data traces in the absence as well as presence of increasing concentrations of $CFTR_{inh}$-172.

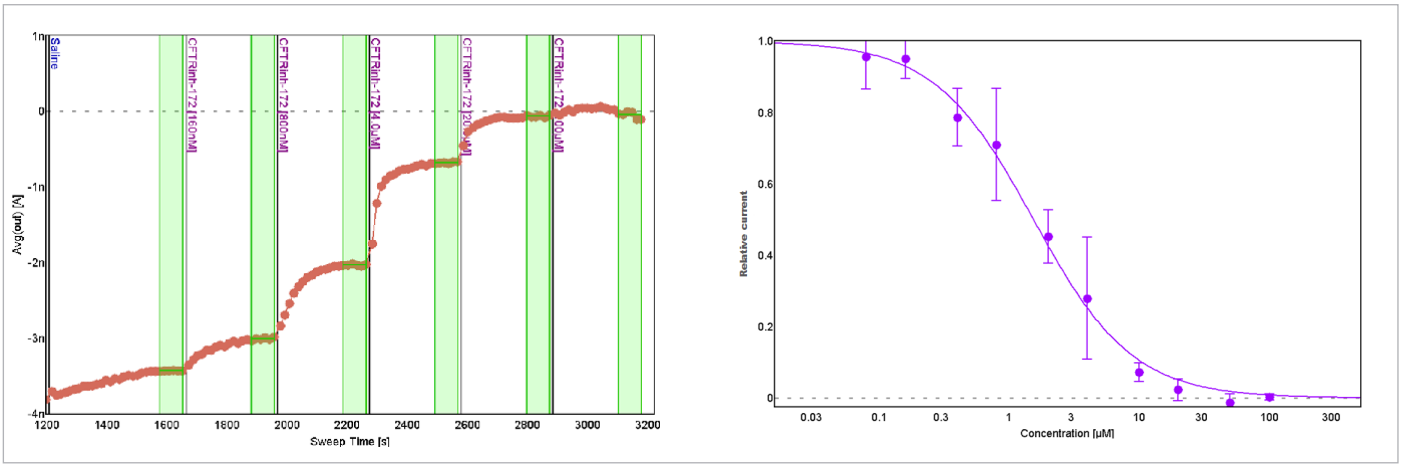

Fig. 2: Five point dose response. Left: Time course of inhibition of the CFTR channel with $CFTR_{inh}$-172. Right: Hill fit for dose response with $CFTR_{inh}$-172, n=18. IC_{50} = 1.55 µM ± 0.19. Error bars are SEM.

The outward 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 aligns well with this site of action (Figure 2). 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). 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). The complete mechanism underlying the block of CFTR-dependent Cl- currents by $CFTR_{inh}$-172 is still not fully understood.

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

Assay

Standard procedures for obtaining whole cell configurations on the QPatch were used.

The voltage protocol used was a ramp from -100 to +100 mV. The voltage protocol was executed every 15 seconds with 1000 Hz sampling frequency and Bessel filtering order 8 (Figure 3). The VP seen in figure 3 was run for at least 5 minutes for every compound addition.

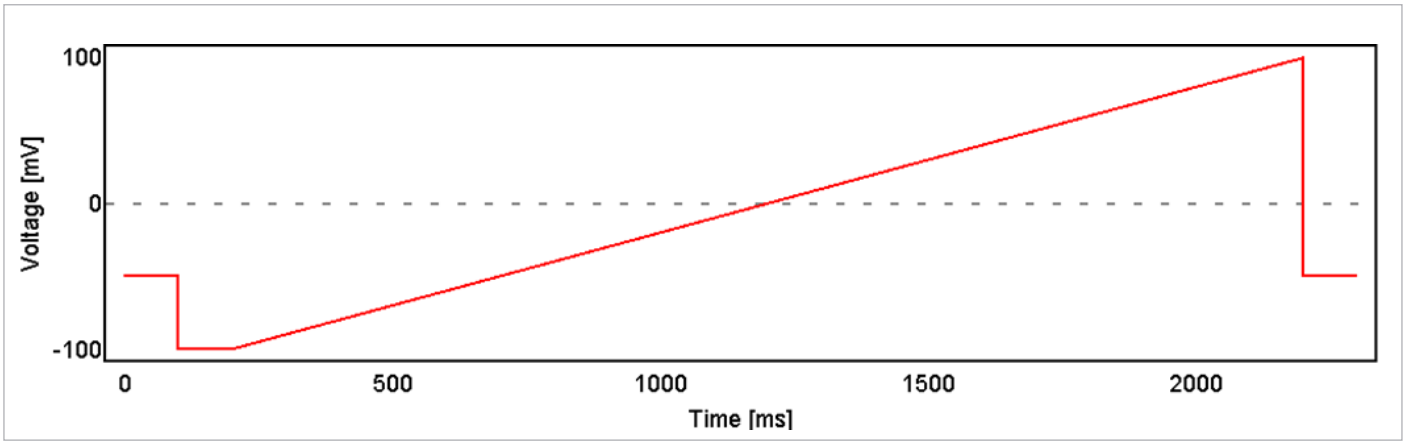

Fig. 3: Voltage ramp from -100 mV to +100 mV from a holding potential of -50 mV.

Materials and Methods

Cell culture: 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 and harvested using Detachin. After detachment cells were kept in serum free media in the QStirrer (cell hotel) until automatic cell preparation immediately prior to assay runs.

Solutions: Intra cellular 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.

Extra Cellular saline solution: 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 5 mM MES, 3 mM KCl, 140 mM NaCl, 20 mM TEA-Cl, pH = 7,3, 290 mOsm.

Seal and whole cell formation: After QPatch automated cell preparation, cell suspension was transferred to the QPlate. A pressure of -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.

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