Application Report:

NIH/3T3 CFTR

Cystic fibrosis transmembrane receptors

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Cystic Fibrosis Transmembrane Receptors

Introduction

NIH/3T3 is a mouse embryonic fibroblast cell line. NIH/3T3 cells are established from NIH Swiss mouse embryos. These cells are highly contact inhibited and are sensitive to sarcoma virus focus formation and leukaemia virus propagation. These cells have now lost their contact inhibition. Contact inhibition is the natural process of arresting cell growth when two or more cells come into contact with each other. The established NIH/3T3 line was subjected to more than 5 serial cycles of subcloning in order to develop a subclone with morphologic characteristics best suited for transformation assays. It is therefore used for DNA transfection studies.

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).

The ΔF508CFTR mutation of the CFTR gene was stably transfected into the NIH/3T3 cell line and used for functional studies on QPatch.

The cell line was supplied to Sophion Bioscience A/S by CombinatorX, Cambridge, MA for initial feasability studies.

Materials & Methods

The cells are grown according to the Sophion standard operating procedure (SOP) for NIH/3T3 cells. NIH/3T3 cells expressing ΔF508CFTR were moved to 27°C 24 hour prior experiments.

Extracellular Ringers solution in mM: 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH 7.4 (with NaOH) and 300 to 305 mOsm. Intracellular in mM: 5.374 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 120 KCl, 4 Na_2-ATP, pH 7.2 (with KOH) and 290 to 295 mOsm.

Assay

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

The voltage protocol used was a ramp from -100 to +100 mV. The voltage protocol was executed every 5 seconds.

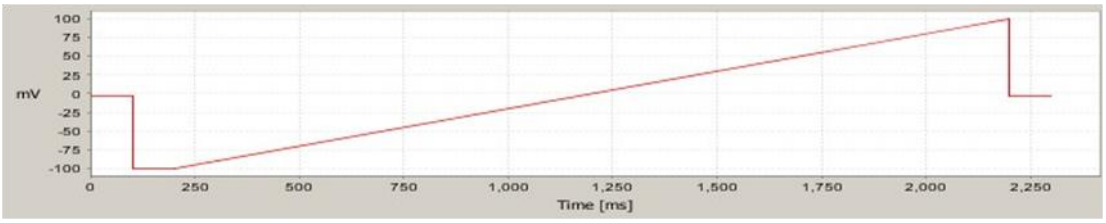

Figure 1. Voltage protocol.

Results

The sealing rate on QPatch was around 70%. The cells were generally large (15-25 pF) and gave stable baseline currents prior to activating the CFTR channels.

QPlate '00465535034807'

Used in job: #2597 - RBJ_NIH/3T3 CFTR -4 mV Glydenclamide DR mideum
Start of use: 2007-08-30 11:59:02

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓				1.74	0.0	0.0	0	0
B1	✓	✓	✓		1.72	146.1	0.0	0	0
C1	✓	✓	✓	✓	1.70	2733.8	273.3	1901	1
D1	✓	✓			1.73	51.2	0.0	0	0
E1	✓	✓	✓	✓	1.65	2211.4	1699.6	1891	1
F1	✓	✓	✓		1.60	1413.5	0.0	0	0
G1	✓	✓	✓	✓	1.81	1658.9	1241.7	197	0
H1	✓	✓	✓	✓	2.12	1366.5	966.2	1888	1
A2	✓	✓	✓	✓	1.64	1001.9	530.2	1858	1
B2	✓	✓	✓	✓	1.66	1255.6	190.5	1854	1
C2	✓	✓	✓	✓	1.58	464.6	1240.7	442	0
D2	✓	✓	✓	✓	1.65	2246.3	144.1	1911	1
E2	✓	✓	✓		1.58	3979.2	0.0	0	0
F2	✓	✓	✓	✓	1.57	1076.2	257.7	1907	1
G2	✓	✓	✓	✓	1.68	3621.4	4364.3	1880	1
H2	✓	✓	✓	✓	1.74	257.6	713.8	1853	1
Total	16	15	14	11					9
Success rate	100 %	94 %	88 %	69 %					

Figure 2. QPlate statistics.

CFTR channels are not voltage sensitive and the channels will stay open when exposed to Forskolin/Genistein. This property makes it difficult to compensate for Cfast, Cslow, Rserie and determinate Rmembrane on QPatch, when performing experiments with different extra cellular Ringers. In experiments performed with the same Ringer pairs throughout the experiment, it was possible to set a holding potential Vhold at ECl (no driving force) and thereby estimate Cfast, Cslow, Rseries and Rmembrane during the experiment.

Forskoline activates adenylat cyclase which use ATP to make cAMP, which then works on the cAMP-dependent protein kinase (PKA). PKA phosphylates the CFTR channel and thereby opens the channel. Genistein prolongs opening bursts and shortens closings.

As can be seen from the screen shot below, the outward Cl-current was more stable than the inward Cl-current. The outward CI-current was thus used for the dose response experiments.

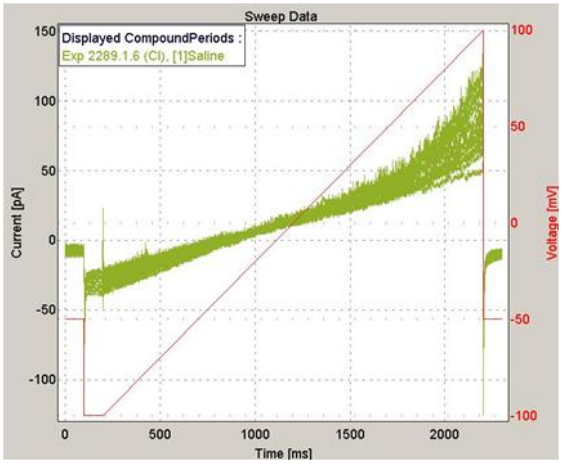

Figure 3. Raw data trace.

Besides chloride, CFTR also conduct a large variety of ions such as fluoride, glutamate, aspartate and gluconate. This makes it difficult to perform ion substitution experiments. By substituting chloride with aspartate (data not shown), gluconate or glutamate, which all have a different permeability than chloride, the results suggest that the inward current was mediated by CFTR.

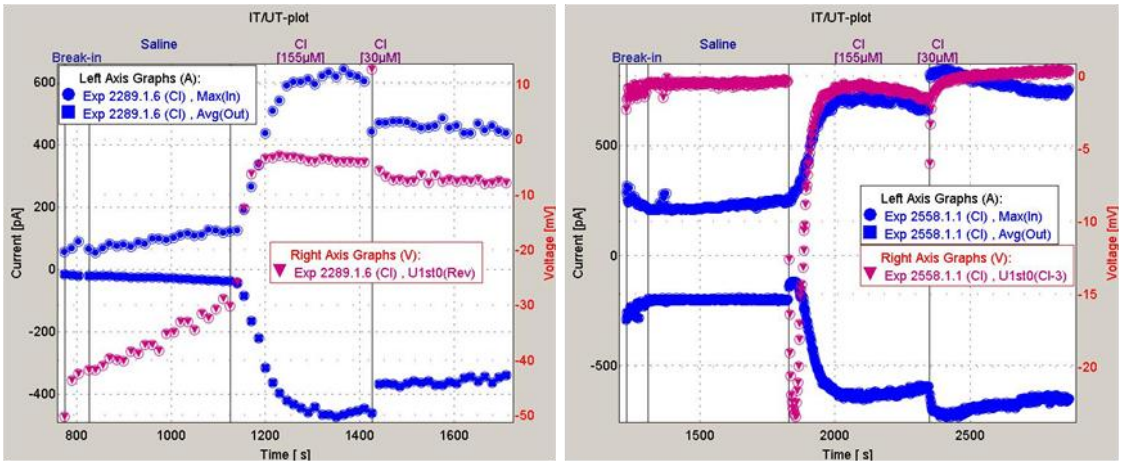

Figure 4. Time vs current/Erev plots. Left: L-Glutamic acid, right: D-Gluconic acid.

The inward and outward currents are seen in the two shots above (blue dots). In the saline period a stable current is obtained in near symmetric chloride (155 EC and 135 IC). In the second period the forskoline/genistein (10/20 µM) mix is added. In the third period the chloride is partly substituted (30 mM EC).

The red dots show the reversal potential determined for each voltage ramp. In the plot to the right a fast and transient change in reversal potential can be seen when forskoline/genistein is added. This change is towards the reversal potential for potassium, EK. We speculate that this is caused by an opening of a cAMP dependent potassium channel.

In a dose response experiment, increased concentrations of glybenclamide were added.

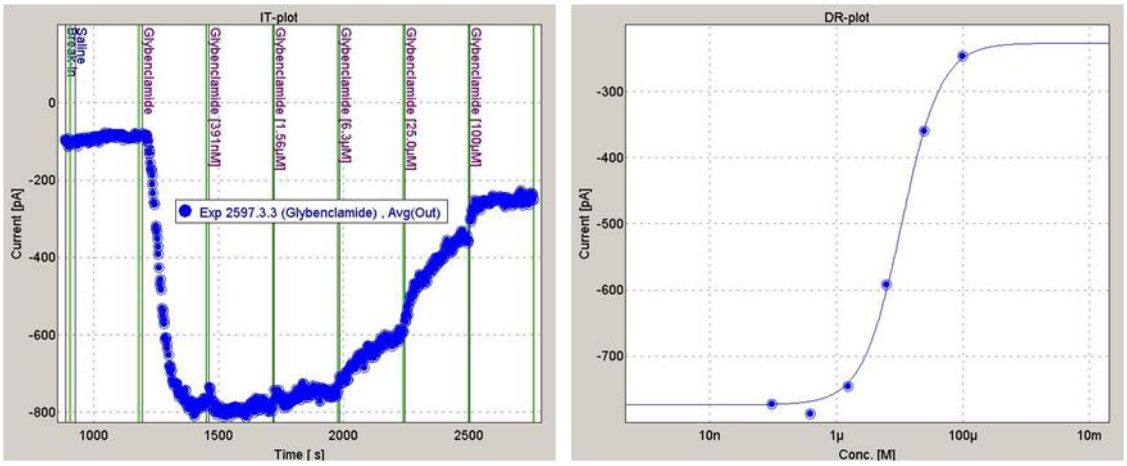

Figure 5. Five point dose response. Left: current vs time plot; Right: Hill fit.

The outward chloride current was mainly mediated by CFTR and could hence be blocked by glybenclamide. IC_{50} for glybenclamide was 26,02 ± 8,15 µM (n=8).

Conclusion

These experiments demonstrate that reliable dose response experiments with the tricky chloride conducting ion channel CFTR can be performed on QPatch. Using different Ringer pairs we identified experimental conditions where specific CFTR activation and closing can be determined.

We conclude that the overall success rates in obtaining stable seals and completed experiments are at a level that clearly identifies the NIH/3T3 – CFTR assay as feasible on QPatch.