

Application Report

NIH/3T3 Cystic fibrosis transmembrane receptors (CFTR) investigated on QPatch

Reliable dose response experiments on CFTR and by using different Ringer pairs we identified experimental conditions where specific CFTR activation and closing could be determined.

Summary

Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel and controls the regulation of other transport pathways. Here we demonstrate that reliable dose-response experiments on the tricky chloride conducting ion channel CFTR can be performed on QPatch. We identified specific activation and closing for CFTR combined with an overall high success rate and stable seals. All in all, CFTR assay is feasible on QPatch.

Introduction

NIH/3T3 is a mouse embryonic fibroblast cell line which is 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 which 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 $\Delta F508$ CFTR mutation of the CFTR gene was stably transfected into the NIH/3T3 cell line and used for functional studies on QPatch.

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.

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [M Ω]	R seal [M Ω]	R whole-cell [M Ω]	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	1365.5	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 %					

Fig. 1. QPatch 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 C_{fast} , C_{slow} , R_{series} and determinate $R_{membrane}$ 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 V_{hold} at ECl (no driving force) and thereby estimate C_{fast} , C_{slow} , R_{series} and $R_{membrane}$ during the experiment.

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

As can be seen from the screen shot in Figure 2, the outward Cl-current was more stable than the inward Cl-current. The outward Cl-current was thus used for the dose-response experiments.

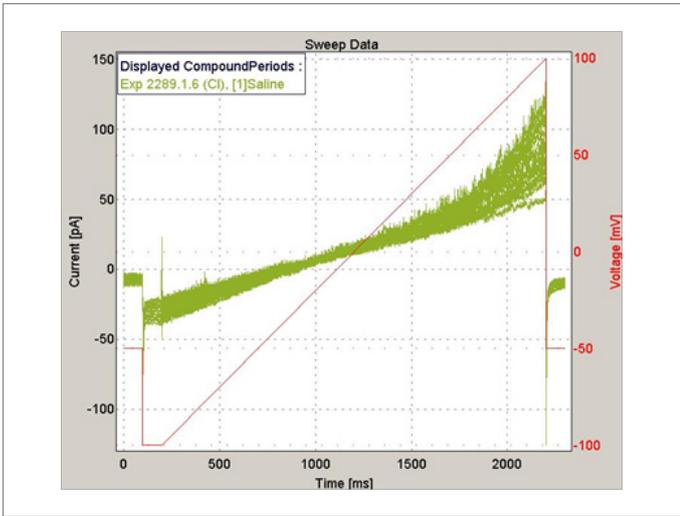


Fig. 2. Raw data trace.

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.

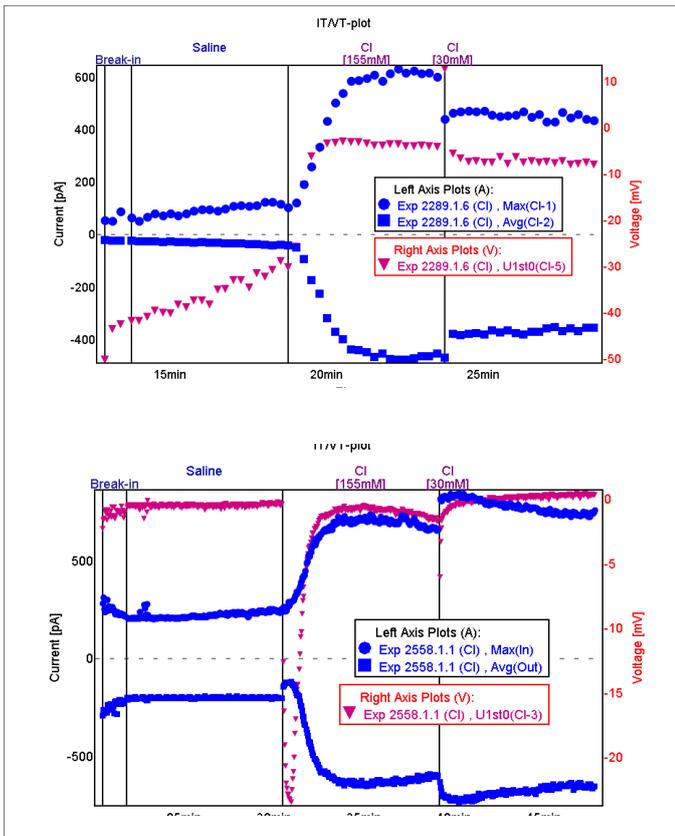


Fig. 3. Time vs. current/Erev plots. Top: L-Glutamic acid, Bottom: D-Gluconic acid.

The inward and outward currents in figure 3 (blue dots). In the saline period a stable current is obtained in near symmetric chloride (155 mM EC and 135 mM IC). In the second period, the forskolin/genistein (10/20 μ M) mix is added and chloride concentrations are kept constant. In the third period, the chloride is partly substituted (30 mM EC).

The red dots in figure 3 show the reversal potential determined for each voltage ramp. In the plot (top panel), a fast and transient change in reversal potential can be seen when forskolin/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 glibenclamide were added.

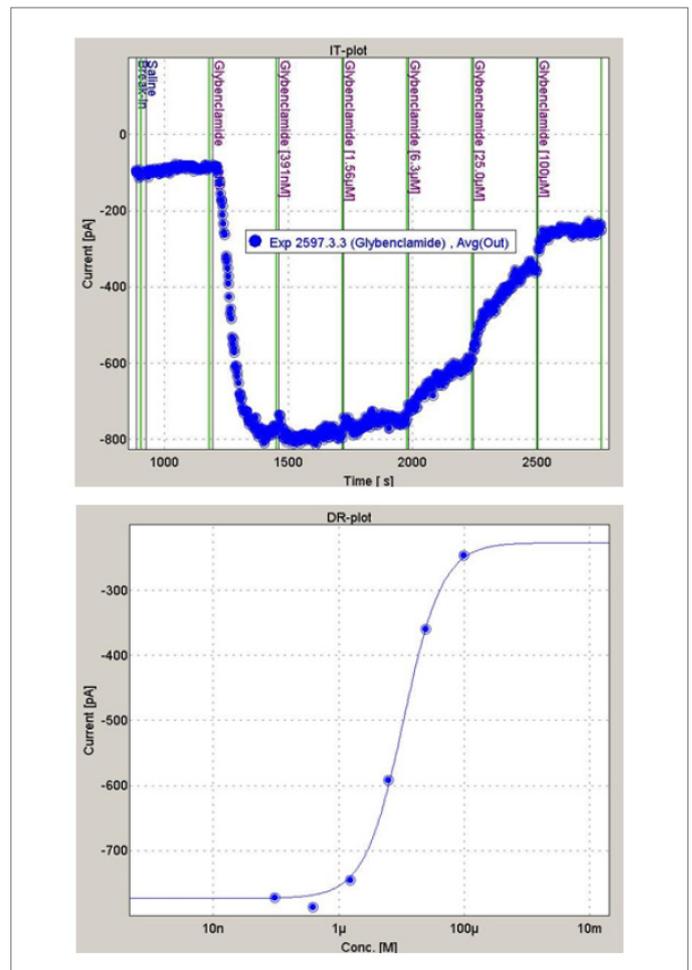


Fig. 4. Five point dose response. Top: current vs time plot; Bottom: Hill fit.

The outward chloride current was mainly mediated by CFTR and could hence be blocked by glibenclamide. IC_{50} for glibenclamide was $26,02 \pm 8,15 \mu\text{M}$ ($n=8$).

Methods

The cells are grown according to the Sophion standard operating procedure and the NIH/3T3 cells expressing $\Delta\text{F508CFTR}$ (supplied by CombinatorX, Cambridge, MA) were moved to 27°C 24 hour prior experiments.

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.

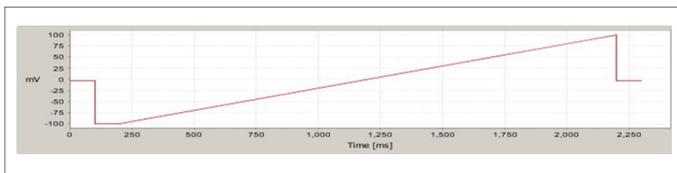


Fig. 5. Voltage protocol

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.