

Different modes of activation of CFTR recorded on Nanion's SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells were kindly provided by Charles River.



Summary

Cystic Fibrosis (CF) is an autosomal recessive genetic disorder which affects a number of organs, in particular the lungs, pancreas and sweat glands. It is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator protein (CFTR)^{1,2}. CFTR is a regulated epithelial chloride channel^{2,3} and mutations cause a reduction in activity of the channel via a variety of mechanisms². This results in defective electrolyte transport in airway epithelia and thereby, chronic lung infection and premature mortality⁴. Therefore, compounds which increase activity of CFTR have therapeutic potential for treating CF.

The CFTR protein is composed of 5 domains: there are 2 transmembrane (TM) domains, 2 nucleotide-binding domains (NBDs) and 1 regulatory domain (R)⁵. The TM domains form the pore of the channel, channel activity is determined by phosphorylation of the R domain and gating is controlled by hydrolysis of ATP at the NBD⁵. CFTR is activated via a number of reagents including internal fluoride⁶, cAMP^{7,8} and external forskolin^{7,8}.

Here we show activation of CFTR expressed in CHO cells on the SyncroPatch 384PE by internal perfusion of F⁻ or external application of forskolin. The current was blocked with the specific blocker, inh-172 with an IC₅₀ in good agreement with the literature⁹. In addition, CFTR activated by internal cAMP was potentiated by VX-770. Using F-free internal solution and activation by submaximal cAMP or forskolin, potentiators of CFTR can be investigated as potential therapeutics to treat CF.

Results

CFTR is activated by internal F⁻, in the presence of ATP⁶, and by external application of forskolin^{7,8}. Figure 1 shows current amplitude at -100 mV or +100 mV versus time for F-free (columns 1 - 12) or F-containing (columns 13 - 24) internal solution on a single plate. In F-free solution, external application of forskolin increased current amplitude. In the presence of internal F⁻, forskolin did not further activate the current.

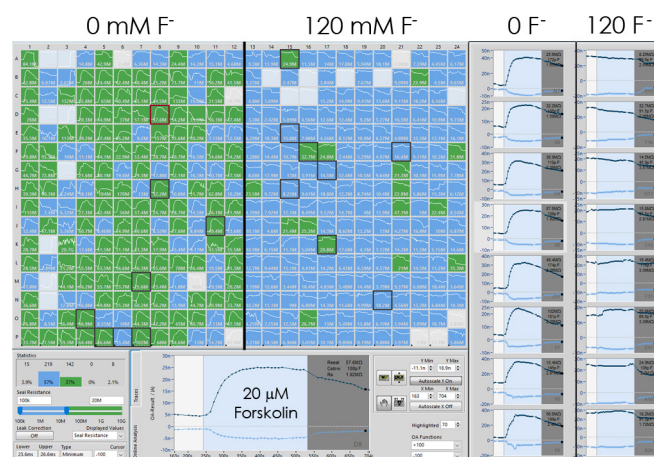


Figure 1: Activation of CFTR using internal F⁻ or external application of Forskolin. Screenshot of the data acquisition software during an experiment. Half the plate (columns 1 - 12) received F-free internal solution and half the plate (columns 13 - 24) had an internal solution containing 120 mM F⁻. The online analysis values of peak current at -100 mV (light blue points) and +100 mV (dark blue points) are shown. Forskolin was applied via the external solution (blue region) which activated CFTR in cells which had F-free internal solution but did not further activate CFTR in cells which had F-containing internal solution. The current was blocked by external application of 150 μM glibenclamide (grey region) regardless of activation method.

Application Note

Currents mediated by CFTR were activated by application of 60 mM F⁻ in the internal solution. The timecourse of activation of the current following internal perfusion of F⁻ could be monitored online and is shown in Figure 2. Raw current traces to a voltage ramp are shown in Panel A. The timecourse of the experiment and the increase in current amplitude at -100 mV and +100 mV upon perfusion of internal F⁻ can be seen in Panel B. The current was blocked by glibenclamide (grey region).

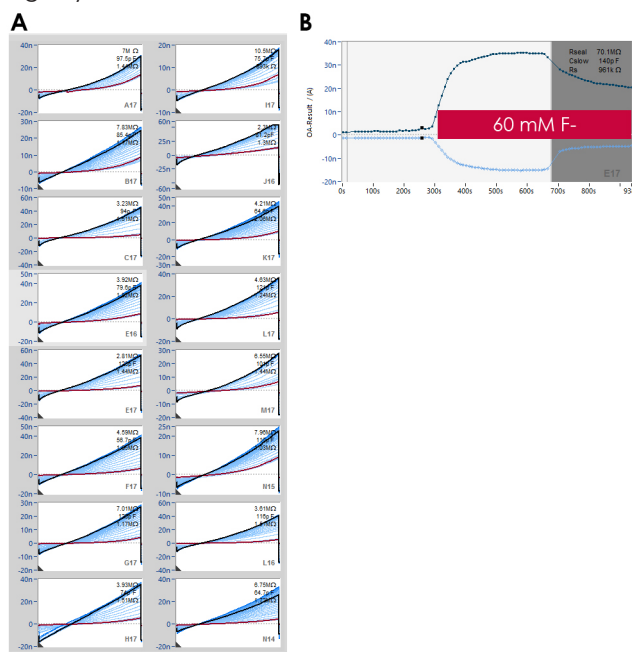


Figure 2: Activation of CFTR by internal perfusion of F⁻. **A** Raw current traces in response to a voltage ramp protocol of 16 example cells are shown. At the beginning of the experiment the internal solution contains 0 mM F⁻ (red trace). CFTR is activated as the internal solution is perfused with F⁻-containing solution (blue and black traces). **B** Online analysis showing current amplitude at +100 mV (dark blue) and -100 mV (light blue) versus time during perfusion of F⁻ in the internal solution. Perfusion of F⁻ is indicated by the red bar. The current was blocked by external application of 200 µM glibenclamide (grey region). This experiment was performed in the whole cell configuration using multi-hole (8X) chips.

CFTR was also activated using internal perfusion of cAMP (Figure 3). 100 µM cAMP was perfused via the internal solution (F⁻-free) and the CFTR-mediated currents were blocked by the specific inhibitor, inh-172. A single-point concentration curve was performed and the average concentration plot was constructed across the plate (Figure 3; half the plate was used for the experiment). The IC₅₀ calculated using a Hill equation was estimated to be 1.6 µM at +80 mV and 1.9 µM at -80 mV in good agreement with the literature using a transport assay⁹ and was voltage independent as expected¹⁰.

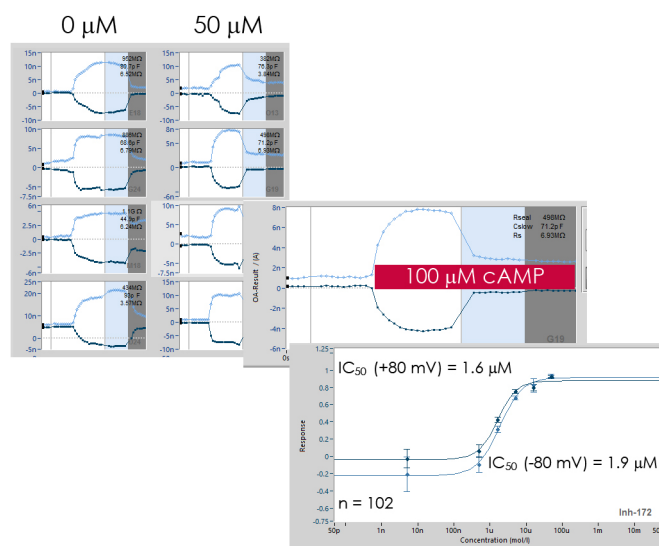


Figure 3: Block of CFTR by inh-172. Shown are online analysis values at +80 mV (light blue) and -80 mV (dark blue) for 8 exemplar cells (top) and one example cell (middle). In this experiment, cAMP perfused internally was used to activate CFTR. The blue region indicates external application of inh-172 and grey is full block with 30 µM inh-172. A concentration response curve was constructed across the plate (half the plate was used for the experiment) and is shown in the bottom panel. The IC₅₀ for inh-172 at +80 mV was 1.6 µM and at -80 mV was 1.9 µM. This experiment was performed in the whole cell configuration using multi-hole (8X) chips.

One potential therapeutic strategy for treating CF is to increase CFTR function in patients with defective CFTR channels due to mutations. VX-770, also known as Ivacaftor, is a CFTR potentiator which increases the activity of wild type and mutant CFTR channels such as ΔF508 and G551D by increasing open probability¹¹. Figure 4 shows activation of CFTR by internal perfusion of cAMP and subsequent potentiation of the response by VX-770. VX-770 potentiated the CFTR response by approximately 25 - 40% at +80 mV and -80 mV, respectively.

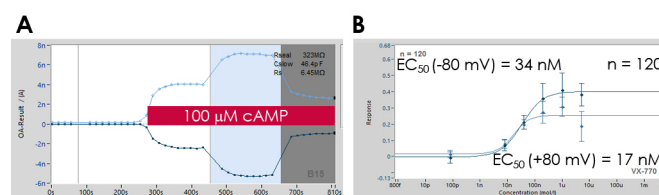
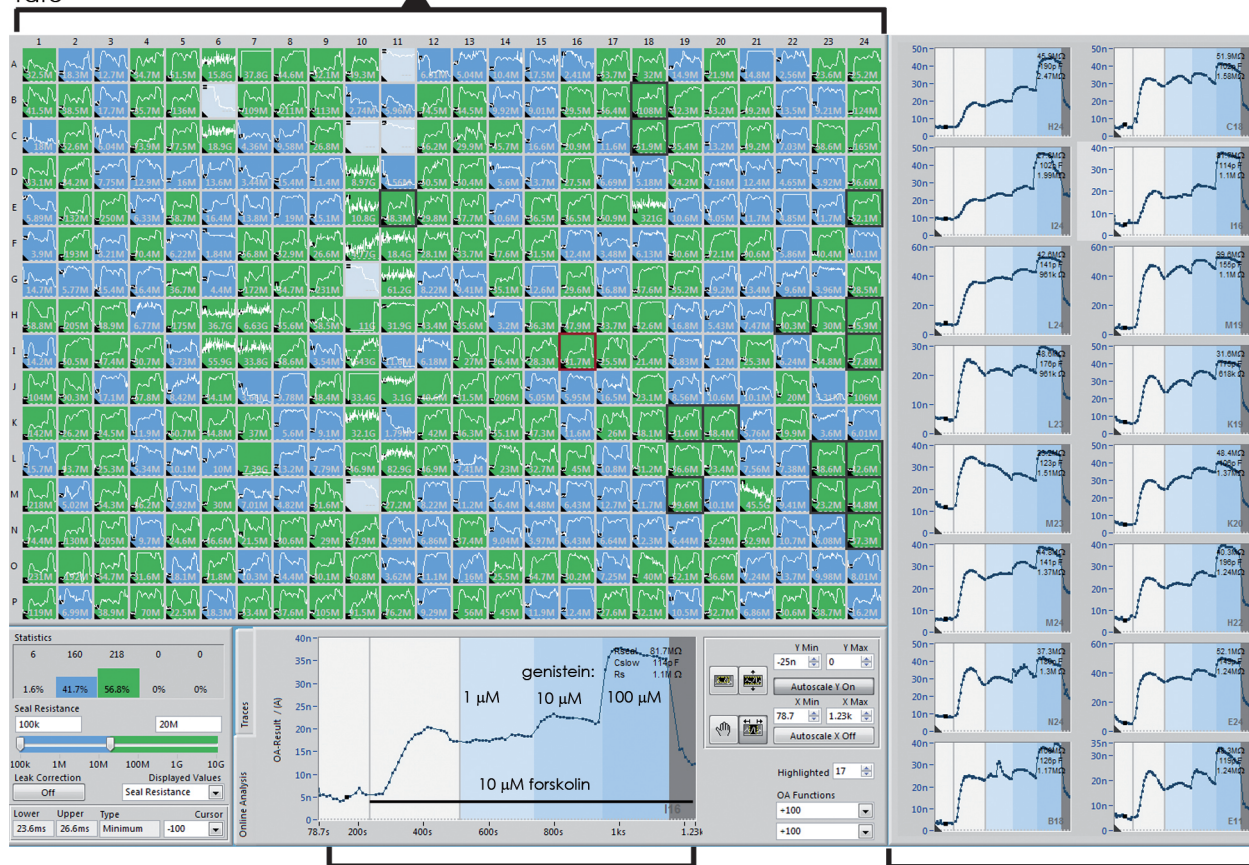


Figure 4: Potentiation of CFTR by VX-770. **A** CFTR was activated using internal perfusion of cAMP. Online analysis values showing current amplitude at +80 mV (light blue) and -80 mV (dark blue) are shown versus time for an example cell. Red bar indicates perfusion of cAMP in the internal solution. Blue region indicates external application of VX-770 (in this example 200 nM) and the grey region indicates external application of 40 µM inh-172 (full block). The concentration response curve was constructed across the plate (half the plate was used for the experiment) and is shown in **B**. VX-770 potentiates the cAMP-induced response by approximately 40% at -80 mV (dark blue) and 25% at +80 mV with an EC₅₀ of 34 nM and 17 nM, respectively.

Application Note

384 color coded depictions of data traces eases judgement of success rate



Highlighted time plot of 1 cell showing activation of CFTR by forskolin and potentiation by genistein

Highlighted time plots of 16 cells showing activation of CFTR by forskolin and potentiation by genistein

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of CFTR expressing CHO cells as recorded on one NPC-384 (multi-hole, 8 holes per well) patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of CFTR activated by external forskolin (10 μM; white region), potentiation by increasing concentrations of genistein (1, 10, 100 μM; blue regions) and full block by 300 μM glibenclamide (grey region).

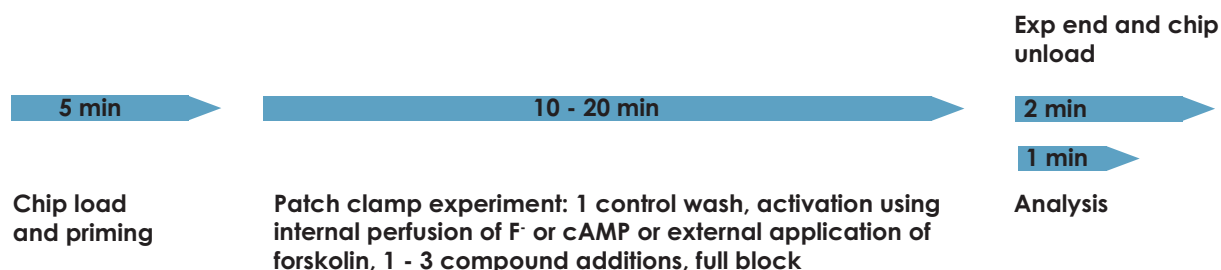


Figure 6: The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single or multiple point concentration response curve plus activation of CFTR with internal F⁻ or cAMP or external application of forskolin and full block at the end of the experiment took approximately 17-27 min.

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Application Note

Figure 5 shows a screenshot of the SyncroPatch® 384 software during an experiment. The user can choose whether to visualize raw traces or online analysis. In this case, online analysis is chosen and the graphs represent current amplitude plotted against time. An individual well can be highlighted to monitor the progression of the experiment. In the Online Analysis view, the time points at which solution additions have been made are indicated by vertical lines, as well as different background colors. In this case, white shows activation of CFTR by external forskolin and the different shades of blue represent increasing concentrations of the potentiator, genistein. Grey shows full block by 300 μ M glibenclamide.

In conclusion, CFTR expressed in CHO cells can be recorded on the SyncroPatch® 384PE with good success rates (typically >80% for RSeal > 500 MOhm at the start of the experiment). The internal perfusion feature of the SyncroPatch 384PE was used to activate CFTR using internal F⁻ or cAMP and increase in the current could be monitored online. This continuous monitoring of current amplitude is important for distinguishing compound effect from increase in

leak or loss of seal and can be a benefit for assay development. CFTR was also activated by external application of forskolin when using a F-free internal solution. The timeline of each experiment was about 17-27 minutes (start – end) and included wash, activation with internal F⁻, cAMP or external forskolin, time to reach a stable current, single or multiple compound concentration(s) application and full block, all performed on each individual well, drastically reducing the consumable cost per data point to < \$0.60. Importantly we could show that CFTR is activated by internal F⁻, internal cAMP or external forskolin as expected^{6,7,8}, inhibited by inh-172 in a voltage-independent manner¹⁰ and potentiated by VX-770¹¹.

The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording CFTR currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell, perfusion of the internal solution and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

CFTR expressing CHO cells, catalog #: CT6113, were supplied by Charles River.

Cell culture

Cells were cultured according to the manufacturer's recommendations and harvested according to Nanion's standard protocol using trypsin. Cells were allowed to grow to confluency and then induced for 24 hours prior to harvesting for experiments.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE using multi-hole (8 holes per well) chips. A voltage ramp protocol from -100 mV to +100 mV over 100 ms was applied to the cells every 10 s. Intersweep holding potential was -20 mV. Current amplitude at +100 mV and -100 mV was used for analysis. Compounds were applied via the internal and external solutions.