Application Note

Ion Channels:
hERG,
$Na_V1.5$
Cells:
HEK-293,
CHO
Tools:
SyncroPatch 384

There is no F in APC: Reliable fluoride-free recordings on the SyncroPatch 384

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Summary

Automated patch clamp (APC) instruments are used for a wide variety of applications ranging from basic research into channelopathies and biophysical characteristics of ion channels, through to routine cardiac safety testing. Their use in cardiac safety screening has increased over the years and APC is now an established and accepted technique in most, if not all, safety testing laboratories. It is well known that fluoride is often used in the internal solution in APC experiments to improve the seal resistance. The presence of external calcium (or other divalent cation) further improves the seal by a mechanism thought to be due to the formation of CaF_2 crystals at the interface between the pipette or micro-pore and the cell as described in a recent patent application [1]. Even in manual patch clamp experiments, fluoride has been used to record voltage-gated Na^+ channels for over 20 years [2,3,4], despite known effects on voltage dependence of conductance, steady-state fast inactivation [4-6] and its inhibition of protein phosphatase [7]. In addition to effects on biophysical properties of ion channels, fluoride binds to calcium making its use in experiments involving activation of ion channels by internal free calcium somewhat limited, and itself activates certain ion channels such as CFTR [8]. It is also well-known that fluoride activates G-proteins when complexed with Al^{3+} present in trace amounts [9]. Fluoride is used because it improves the seal and allows stable measurements to be performed over long periods of time [10,11]. However, because there are some experiments where it is advantageous to use physiological, fluoride-free internal solutions and external solution that does not use divalent 'seal enhancer' solutions, we have developed a method that allows fluoride-free, physiological solutions to be used with good success rates. We demonstrate this using the cardiac ion channels hERG expressed in HEK293 cells (SB Drug Discovery) and $Na_V1.5$ expressed in CHO cells (Charles River).

Results

hERG expressed in HEK293 cells were recorded on the SyncroPatch 384 using fluoride-free (FF) internal solution (Figure 1A) or standard (fluoride-containing) internal solution (Figure 1B). For hERG, a classical double voltage-step protocol was used to record hERG tail currents and we determined the number of wells available for recording under both conditions. Little difference in current amplitude (Figure 1C) and R_{Seal} (Figure 1D) was observed between the two groups.

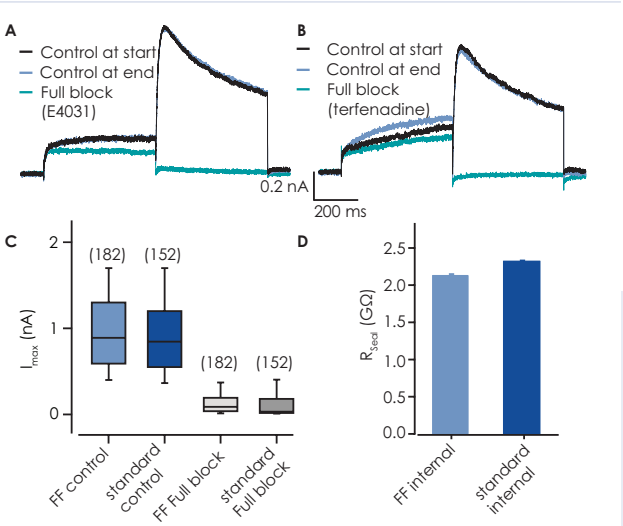


Figure 1: A *hERG currents from an example cell recorded using FF internal solution.* **B** *hERG currents recorded from an example cell using standard internal solution.* **C** *Box plot of current amplitudes for an example NPC-384 chip in FF and standard internal solution and after block with E4031 or terfenadine* **D** *Bar graph of R_{Seal} values for one experiment for wells for FF and standard internal (mean ± SEM).*

Current amplitude was also stable over time and hERG-mediated currents were almost identical at the end of the recording compared with the start (Figure 1). A single addition of compound per well was performed and at the end of the experiment, a full block concentration was used. The hERG current was completely inhibited by a full block concentration of either E4031 or terfenadine (Figure 1).

Success rates (% available wells) were calculated for multiple NPC-384 chips using either standard internal solution or FF internal solution and no use of 'seal enhancer'. Although success rates were better when fluoride was used, as expected, success rates of 34 ± 8% (n = 32 chips) were achieved without fluoride for wells with R_{Seal} ≥ 1GΩ at the end of the experiment, and this increased to 57 ± 9% if the quality control parameter R_{Seal} ≥ 0.25 GΩ at the end of the experiment was used, giving a success rate of almost 60% for completed experiments. There was little loss of wells over time, the success rate at the end of the experiment was similar to that at the start (Figure 2A,B).

We also used CHO cells expressing the cardiac $Na_V1.5$ channel. Using these cells, success rates for R_{Seal} ≥ 1 GΩ were 42 ± 9% in FF versus 78 ± 5% in standard solution (Figure 2C) were achieved. This increased to a success rate of 55 ± 12% for FF if the quality control parameter R_{Seal} ≥ 0.25 GΩ was used and 86 ± 6% for standard solution (Figure 2D), a cutoff which indicates an acceptable R_{Seal} for screening. There was little loss of wells over time, the success rate at the end of the experiment was similar as at the start.

$Na_V1.5$-mediated currents were stable over time in both FF internal solution or standard internal solution (Figure 3) and completely inhibited by a full block concentration of tetracaine (333 µM).

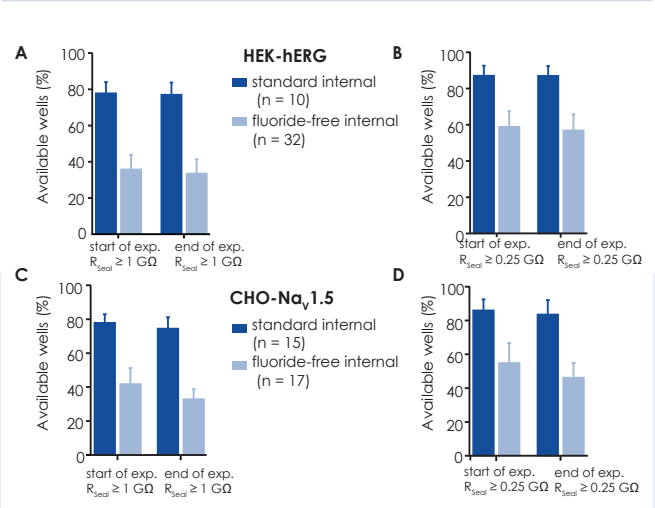


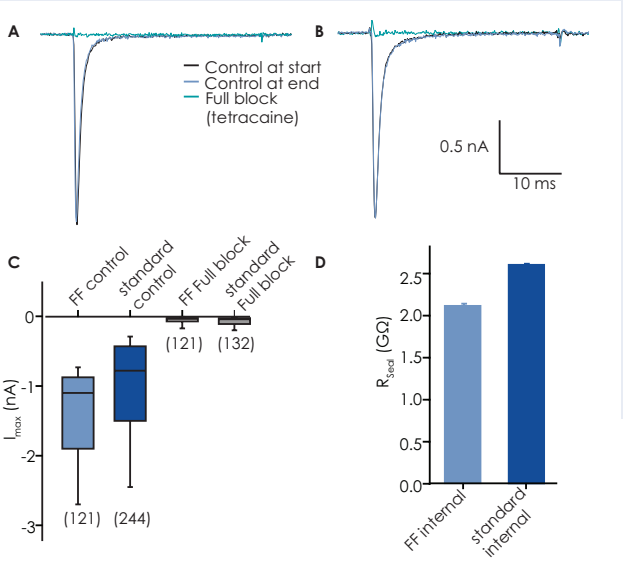
Figure 2: *Success rates for HEK and CHO cells in the presence and absence of fluoride. Shown are bar graphs for number of successful wells for HEK-hERG cells R_{Seal} ≥ 1 GΩ (**A**) or R_{Seal} ≥ 0.25 GΩ (**B**) and for CHO-$Na_V1.5$ cells where R_{Seal} ≥ 1 GΩ (**C**) or R_{Seal} ≥ 0.25 GΩ (**D**).*

We were also interested in investigating the effects of internal fluoride on the V_{half} of activation and inactivation of $Na_V1.5$ channels because it has been reported previously that fluoride affects these parameters in other Na_V channels [4-6,12]. There is a relatively large variability in V_{half} values reported for $Na_V1.5$ in the literature, ranging from -25 mV [13] to -50 mV [14]. This is presumably due to different experimental parameters including voltage protocol, recording solutions, co-expression with β-subunits, recording temperature, holding potential, or cell expression system. For this reason, we kept all experimental parameters the same, and only changed the internal solution in order to examine only the effect of fluoride on the V_{half} of activation and inactivation of $Na_V1.5$. Our results show that fluoride in the internal solution causes a negative shift in the V_{half} of activation and inactivation of $Na_V1.5$. Figure 4 shows the activation and inactivation curves of $Na_V1.5$. for both conditions (standard or FF internal solution). The V_{half} of activation was -25.2 ± 0.5 mV (n = 241) for FF and -37.5 ± 0.4 mV (n = 484) for standard internal solution. A similar difference was observed for the V_{half} of inactivation with -53.6 ± 0.6 mV (n = 165) in FF and -68.4 ± 0.4 mV (n = 728) using standard internal. This agrees with previous reports that fluoride shifts the V_{half} of activation and inactivation to more negative values of $Na_V1.3$ [12], $Na_V1.7$ [5], and $Na_V1.9$ [4,6]. The values for V_{half} of activation and inactivation for $Na_V1.5$ were in good agreement with the literature [13-16].

To test whether fluoride influences pharmacology of $Na_V1.5$ we used the known Na_V channel blocker, tetracaine. This was applied as a single concentration of compound to each well, followed by a full block concentration (333 µM). Using a holding potential of -120 mV, the concentration response curves for tetracaine in standard internal or FF internal solution almost exactly overlaid (Figure 5), and the IC_{50} values for tetracaine were not statistically significantly different (Student's t-test, P > 0.05) for 4 NPC-384 chips in standard internal and 3 NPC-384FF chips in FF internal.

As expected,the potency of inhibition increased with a more depolarized holding potential (-100 mV or -80 mV) for both conditions, consistent with local anesthetics preferably binding to the inactivated states [17-20] (data not shown, for full data set please see Ref. 21).

Figure 3: A *$Na_V1.5$-mediated currents recorded on the SyncroPatch 384 using FF internal solution or (***B***) standard internal solution.* **C** *Box plot of current amplitudes for an example NPC-384 plate in FF and standard internal solution. The current amplitude after block with tetracaine is also shown.* **D** *Bar graph of R_{Seal} values for one experiment for 121 and 244 wells for FF and standard internal, respectively. Shown are mean ± SEM.*



Conclusions

The SyncroPatch 384 is a high throughput and highly reliable automated patch clamp device which can be used with standard (fluoride-containing) internal solution for high success rates and long-lasting recordings. Alternatively, fluoride-free physiological internal solution (and no external seal enhancer) can be used with good success rates, making it possible to perform high throughput APC recordings in physiological solutions used in manual patch clamp. We have overcome the challenge of low success rates and low seal resistances on high throughput automated patch clamp devices when using fluoride-free physiological internal solution by implementing the use of specialized consumables (NPC-384FF). This has enabled us to report success rates for fluoride-free recordings of approximately 60–80% for R_{Seal} ≥ 0.25 GΩ and 40–50% for R_{Seal} ≥1 GΩ.

We tested different cell lines and found that similar success rates were achieved for CHO and HEK293 cells using FF internal solution. Little difference was observed for hERG currents, however, in experiments involving Na_V channels, we confirmed a hyperpolarizing effect of fluoride on V_{half} of activation and inactivation. Although this did not affect IC_{50} of tetracaine, users should be aware that fluoride causes shifts in activation and inactivation parameters and caution should be used when comparing data generated in different solutions.

Our results further highlight the flexibility of the SyncroPatch 384 which can be used as a high throughput screening device in standard, fluoride-containing internal solution, but also for smaller screening projects taking advantage of the 32-well mode of operation, or the use of physiological fluoride-free internal solutions which may be important for certain experiments or for comparison of data to manual patch clamp experiments.

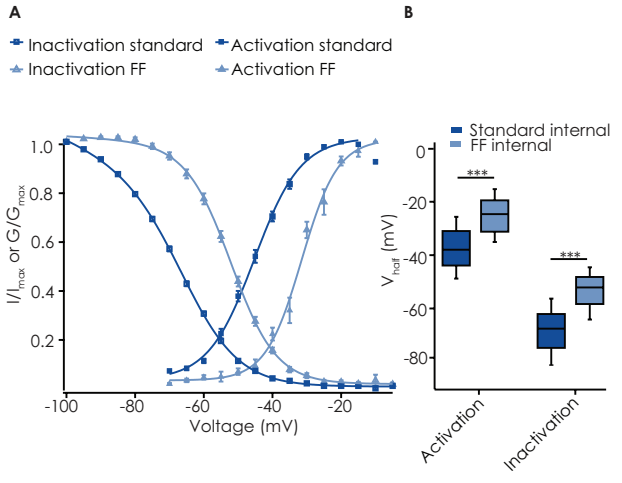


Figure 4: A *Activation and inactivation IV curves for $Na_V1.5$ in standard internal and FF internal solution are shown overlaid.* **B** *The V_{half} values of activation of 484 cells in standard internal were significantly more negative compared with 241 cells in FF internal. The same shift to more negative V_{half} of inactivation was also found for 728 in standard internal fluoride compared with 165 cells in fluoride-free internal (Student's t test, ***P < 0.001).*

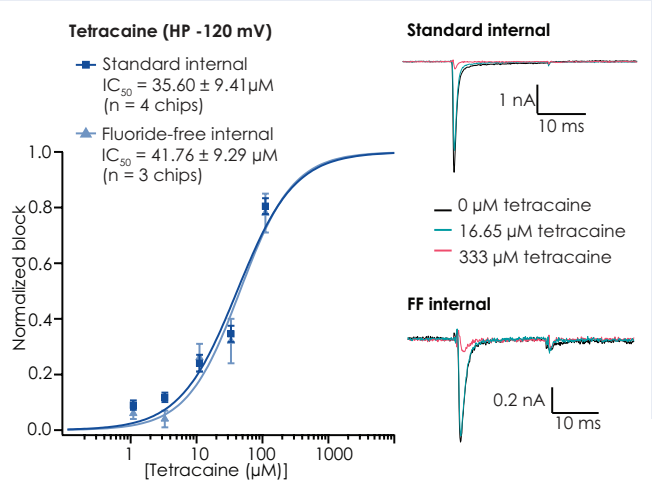


Figure 5: *Pharmacology of $Na_V1.5$ recorded in standard and FF internal solution. Concentration response curves for tetracaine in standard internal solution or FF internal solution at a holding potential of -120 mV are shown overlaid. Example traces in standard and FF internal solution in the presence of tetracaine are also shown.*

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Methods

Cells

HEK-293 cells expressing hERG (SB Drug Discovery) or CHO cells expressing $Na_V1.5$ (Charles River) were used.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384. Cells were resuspended in external recording solution and kept in the CellHotel of the SyncroPatch 384 at 12°C prior to pipetting into the NPC-384 or NPC-384FF chips for the experiment. hERG was recorded using a double step voltage protocol from a holding potential of -80 mV to +60 mV for 500 ms followed by a step to -40 mV for 500 ms repeated every 15 s. Peak amplitude was measured at the start of the second voltage step. $Na_V1.5$ was measured using a single step protocol from -120 mV to 0 mV repeated every 10 s. Standard internal solution contained 110 mM KF, fluoride-free internal solution contained 110 mM K-gluconate. External solution contained 60 mM NMDG. Please ask for more details.

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