Development and Evaluation of Novel Solution Pairs to Enhance Seal Resistance in Automated Patch Clamp Experiments

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Introduction

Gigaohm seals, or 'gigaseals', are crucial for patch clamp electrophysiology, ensuring excellent electrical access to cells to enable high-quality recordings. These seals form through chemical bonds and electrostatic forces between the cell membrane and the glass pipette in manual patch clamp, or between the cell membrane and chip substrate in planar patch clamp. Planar patch clamp often employs 'seal enhancers' to increase seal resistances, with CaF_2 being the most commonly used. It is hypothesized that high extracellular Ca^{2+} and intracellular F^- concentrations lead to CaF_2 precipitate formation at the solution interface, promoting seal formation.However, CaF_2 as a seal enhancer has limitations. F^- interacts with various internal components such as protein kinase A, adenylate cyclase, and lipid phosphatases, which can affect the biophysical properties of some ion channels. Additionally, F^- is not ideal when recording from Ca^{2+}- activated ion channels due to the resulting unknown concentrations of free intracellular Ca^{2+}.

In an effort to overcome these limitations, Sophion developed new solution pairs in 2017 that foster seal formation (Patent: WO2018100206A1). Building on this technology, Metrion and Sophion collaborated to further determine whether other insoluble salts can act as seal enhancers and how these solution pairs affect the biophysical properties and pharmacology of the investigated ion channels.

Conclusion

- $BaSO_4$ was identified as an equivalent seal enhancer to CaF_2.
- The two solution pairs were characterized across two ion channels: $hNa_V1.5$ and $hCa_V1.2$.
- Intracellular F^- caused depolarizing shifts in the voltage dependence of inactivation of $hNa_V1.5$ where no such effects were observed with SO_4^{2-} in the intracellular solution.
- No difference in pharmacological effects of inhibitory compounds against $hNa_V1.5$ or $hCa_V1.2$ was observed between the two solution pairs, CaF_2 and $BaSO_4$.
- $BaSO_4$ is well-suited as a seal enhancer for recording from non-K^+-conducting Ca^{2+}-activated channels, such as TMEM16A. In particular as $BaSO_4$ allows more accurate estimation of free intracellular Ca^{2+} concentration.

Material and methods

Experiments were conducted at 22°C using a Sophion Bioscience Qube 384 with QChip 384 (single hole) and QChip 384X (multihole) consumables.

Analysis was conducted using Sophion Analyzer v9.0.42 and GraphPad Prism v10.2.2.

CHO-$hNa_V1.5$ and HEK293-$hCa_V1.2$ cell lines were provided by Metrion Biosciences.

All compounds were tested at: 0.001, 0.01, 0.1, 1, 10 and 100 µM.

Results

Table 1: Correlation between salt pair solubility product constants (K_{sp}) and gigaseal formation on Qube 384. No correlation was found between the solubility product constants (K_{sp}) of Ca^{2+}, Ba^{2+} and Sr^{2+} salts **(A)** and their ability to foster gigaseal formation **(B)**. Despite the PO_4^{3-} salts having very low K_{sp} values and $SrCO_3$ having a similar K_{sp} value to CaF_2 and $BaSO_4$, these salts failed to produce gigaohm seals. Moderate seal resistances with PO_4^{3-} salts were transient and unstable. Median resistances calculated from 24 cells per salt pair.

A.

K_{sp}	Solubility product constant (K_{sp}) at 25°C; pH7.0				
	F^-	SO_4^{2-}	CO_3^{2-}	PO_4^{3-}	$C_2O_4^{2-}$
Ca^{2+}					
Ba^{2+}					
Sr^{2+}					

1 x 10^{-5} Sparingly soluble — 1 x 10^{-33} Insoluble

B.

GΩ		Median $R_{membrane}$ (GΩ)				
		120 mM F^-	90 mM SO_4^{2-}	90 mM CO_3^{2-}	70 mM PO_4^{3-}	90 mM $C_2O_4^{2-}$
10 mM	Ca^{2+}					
	Ba^{2+}					
	Sr^{2+}					

0 MΩ — > 1 GΩ

Table 2: Resistances of seals formed using various concentrations of extracellular Ba^{2+} and intracellular SO_4^{2-}. Gigaseals only formed with ≥ 3 mM Ba^{2+} and in the presence of SO_4^{2-}. Median resistances calculated from 24 or 48 cells per condition.

CHO-$hNa_V1.5$	Median $R_{membrane}$ (GΩ)			
Single-hole experiments	0 mM SO_4^{2-}	50 mM SO_4^{2-}	70 mM SO_4^{2-}	90 mM SO_4^{2-}
Ba^{2+} (mM) 0				
0.01				
0.03				
0.1				
0.3				
1				
3				
5				
10				

0 MΩ — > 1 GΩ

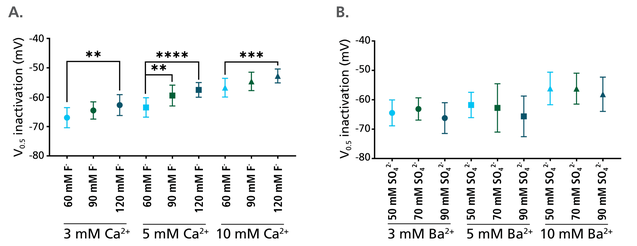
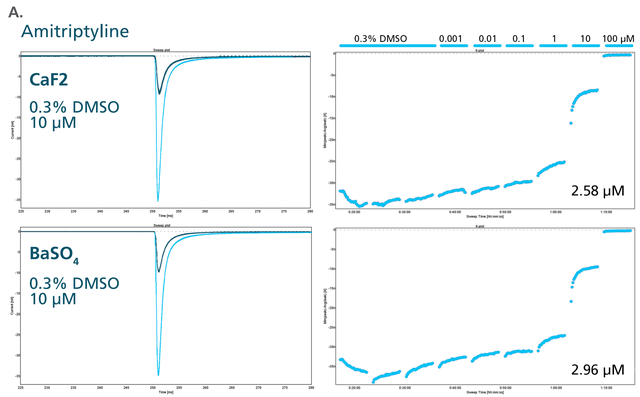


Fig. 1: Effects of CaF_2 and $BaSO_4$ on $hNa_V1.5$ channel biophysics. $hNa_V1.5$ $V_{0.5}$ inactivation with different cation and anion concentrations (mean ± S.D.; N ≥ 11). Increasing concentrations of intracellular F^- caused a depolarizing shift in $V_{0.5}$ inactivation **(A)**. In contrast, increasing concentrations of SO_4^{2-} had no effect on $hNa_V1.5$ $V_{0.5}$ inactivation **(B)**. One-way ANOVAs conducted within each cation group followed by Tukey's Honestly Significant Difference post-hoc tests: ** = $p < .01$; *** = $p < .001$; **** = $p < .0001$.



B.

CHO- $hNa_V1.5$	IC_{50} (µM)	
Multihole experiments	3 mM Ca^{2+} + 120 mM F^-	50 mM Ba^{2+} + 70 mM SO_4^{2-}
Amitriptyline	2.6	3.0
Tetracaine	3.7	5.5
Bepridil	12.0	14.0
Verapamil	16.4	18.0
Dapoxetine	9.1	11.6
Lidocaine	>100	>100
Phenytoin	>100	>100

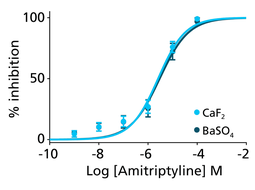


Fig. 2: CaF_2 versus $BaSO_4$ – $hNa_V1.5$ pharmacology. (A) Representative sweep plots (left) and current-time (I-t) plots (right) for $hNa_V1.5$ inhibition by amitriptyline. There was no difference in cumulative inhibition of $hNa_V1.5$ by increasing concentrations of amitriptyline between CaF_2 and $BaSO_4$. **(B)** Screening of a range of inhibitory compounds showed no difference in $hNa_V1.5$ pharmacology between CaF_2 and $BaSO_4$. Concentration-response curves for amitriptyline against $hNa_V1.5$ using CaF_2 or $BaSO_4$ as the seal enhancer (mean ± S.D.; N = 12 wells per concentration for CaF_2, N = 8 wells per concentration for $BaSO_4$).

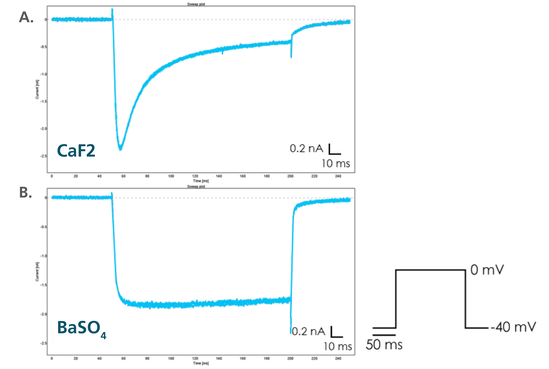


Fig.3: CaF_2 versus $BaSO_4$ – $hCa_V1.2$ kinetics. $hCa_V1.2$ exhibits Ca^{2+}-dependent inactivation when CaF_2 is used as the seal enhancer **(A)**. $BaSO_4$ as the seal enhancer (using Ba^{2+} as a surrogate carrier ion) **(B)** confers loss of the Ca^{2+}-dependent inactivation of $hCa_V1.2$ observed with CaF_2. Example sweep plots derived from Sophion Analyzer v9.0.42.

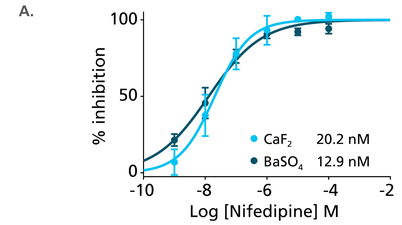


Fig.4: CaF_2 versus $BaSO_4$ – $hCa_V1.2$ pharmacology. Mean ± S.D. concentration-response curves for two common inhibitors against $hCa_V1.2$, nifedipine **(A)** and verapamil **(B)** (CaF_2: N = 2-5 wells per concentration; $BaSO_4$: N = 6-12 wells per concentration). Compound potencies (IC_{50} values) did not differ between CaF_2 and $BaSO_4$.

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