Seal enhancers on the Qube 384: An alternative to F^-

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Introduction

Gigaohm seals, or 'gigaseals', are imperative to patch clamp electrophysiology to enable good electrical access to the cell and 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 in the case of planar patch clamp, between the cell membrane and chip substrate[1]. Planar patch clamp often requires the use of 'seal enhancers' to increase the resistances of these seals, with CaF_2 being the most extensively used. It is hypothesised that high concentrations of extracellular Ca^{2+} and intracellular F^- give rise to CaF_2 precipitate at the solution interface, fostering seal formation[2].

CaF_2 as a seal enhancer, however, has limitations. F^- is known to stimulate G-protein modulation of ion channels, altering channel properties[3-6]. Furthermore, use of F^- is not optimal when recording from Ca^{2+}-activated ion channels due to resultant unknown concentrations of free intracellular Ca^{2+}.

In an effort to overcome these limitations, Metrion and Sophion collaborated to determine whether other insoluble salts can act as seal enhancers, or whether this property is unique to CaF_2.

Results

The solubility product constants (K_{sp}) of different Ca^{2+}, Ba^{2+} and Sr^{2+} salts are compared in Table 1a. $BaSO_4$ and $SrCO_3$ have low K_{sp} values (with corresponding low solubility), similar to CaF_2. The PO_4^{3-} salts, however, have even lower K_{sp} values and are highly insoluble. The ability of the different salt pairs to promote gigaseal formation was analysed using a CHO-h$Na_v1.5$ cell line and single hole QChips. The cells were resuspended in extracellular solutions containing 10 mM Ca^{2+}, Ba^{2+} or Sr^{2+}, whilst a selection of intracellular solutions was used containing high concentrations of the different anions. The concentration of each anion was adjusted to ensure consistent osmolarity across the intracellular solutions (~290 mOsm). Only $BaSO_4$ successfully facilitated the formation of gigaseals comparable to CaF_2 (Table 1b). Interestingly, these data suggest there is no correlation between salt K_{sp} values and their ability to foster gigaseal formation, particularly as neither $SrCO_3$ nor the PO_4^{3-} salts significantly increased seal resistances. Thus, the relationship between salt crystal formation and gigaseals remains unclear. Still, as SO_4^{2-} is not known to alter ion channel function or cellular signalling pathways, $BaSO_4$ represents a candidate alternative seal enhancer to CaF_2 with potential advantages.

Experiments were subsequently conducted to determine the minimal concentration of extracellular Ba^{2+} required for the formation of gigaseals. To this end, CHO-h$Na_v1.5$ cells were resuspended in extracellular solutions containing different concentrations of Ba^{2+}, and seals formed in the presence of different levels of intracellular SO_4^{2-}. High resistance gigaohm seals only formed with ≥ 3 mM extracellular Ba^{2+} (Table 2). It is known, however, that Ba^{2+} ions block K^+ channels in the µM-mM range[7-9]. Thus, whether this concentration is low enough to spare K^+ channels from block is yet to be determined.

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)**. Only $BaSO_4$ successfully facilitated the formation of gigaseals comparable to CaF_2. 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. BaF_2 and SrF_2 were not studied as alternative seal enhancers to CaF_2 as they do not eliminate the use of F^-. Median resistances calculated from 24 cells per salt pair.

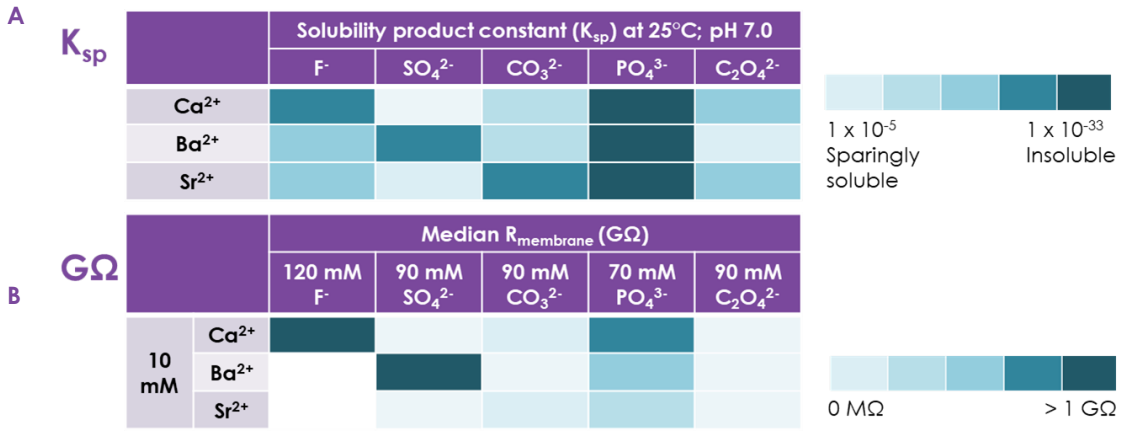
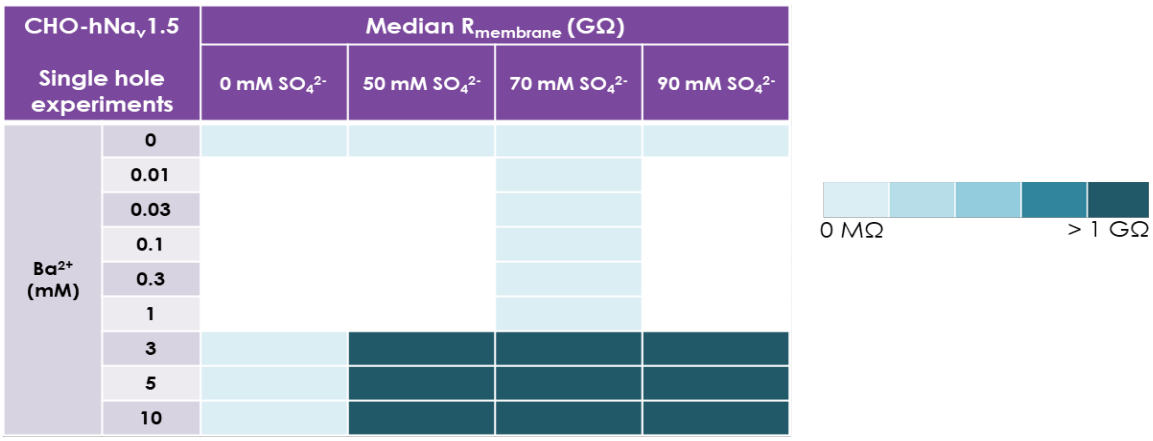


Table 2. Resistances of seals formed using various concentrations of extracellular Ba^{2+} and intracellular SO_4^{2-}. Gigaseals only formed with ≥ 3 mM Ba^{2+} and in the presence of SO_4^{2-}. Median resistances calculated from 24 or 48 cells per condition.



Effects of CaF_2 and $BaSO_4$ on $hNa_v1.5$ biophysical properties

F^- is known to trigger G-protein modulation of ion channels and has been reported to affect the voltage-dependent kinetics of Na_v channels[4-6, 10-13]. Single hole experiments were conducted to assess the effects of CaF_2 and $BaSO_4$ as seal enhancers on $hNa_v1.5$ biophysical properties.

CHO-$hNa_v1.5$ cells were resuspended in extracellular solutions containing 3, 5 or 10 mM Ca^{2+} or Ba^{2+}, and seals formed in the presence of intracellular solutions containing various concentrations of F^- or SO_4^{2-}. A series of 3-s pulses between -100 mV and +50 mV in +10 mV intervals from a holding potential of -120 mV, followed by a 20-ms pulse at 0 mV was used to determine the voltage dependence of activation and inactivation of $hNa_v1.5$. There was a significant depolarising shift in $hNa_v1.5$ $V_{0.5}$ inactivation with increasing concentrations of intracellular F^- (Figure 1a). In contrast, increasing SO_4^{2-} concentration had no significant effect on $V_{0.5}$ inactivation (Figure 1b). This suggests that, unlike the G-protein effects of F^- on $hNa_v1.5$, SO_4^{2-} has no effect on intracellular signalling and does not impact channel biophysics. Of note, there was also a depolarising shift in $hNa_v1.5$ $V_{0.5}$ inactivation with higher concentrations of extracellular Ca^{2+} and Ba^{2+} most likely due to surface potential screening effects (independent of G-protein modulation)[14-15]. In contrast to the voltage dependence of inactivation, there was no significant difference in $hNa_v1.5$ current amplitude or $V_{0.5}$ activation between the two seal enhancers at the different concentrations (data not shown).

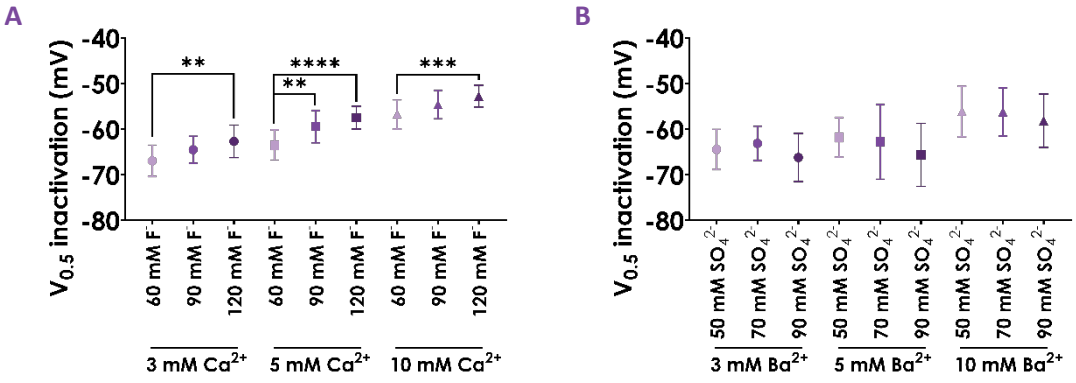
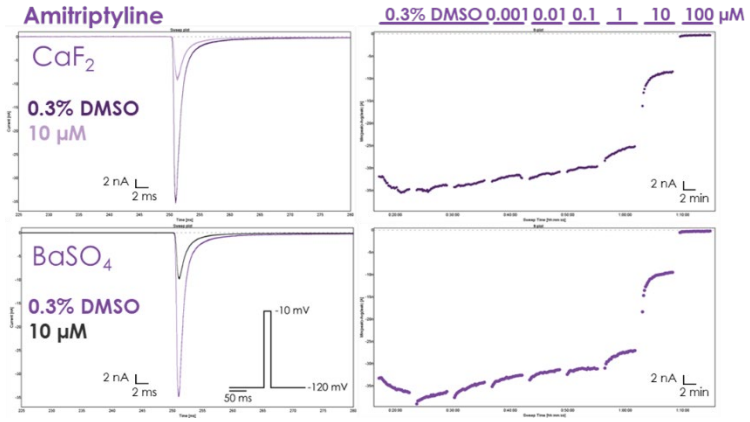


Figure 1. Effects of CaF_2 and $BaSO_4$ on $hNa_v1.5$ channel biophysics. $hNa_v1.5$ $V_{0.5}$ inactivation with different cation and anion concentrations (mean ± S.D., N ≥ 11). Increasing concentrations of intracellular F^- caused a depolarising shift in $V_{0.5}$ inactivation **(A)**. In contrast, 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: ** = $p < .01$; *** = $p < .001$; **** = $p < .0001$. CaF_2 and $BaSO_4$ data taken from two separate experiments.

hNa$_v$1.5 pharmacology: CaF_2 versus $BaSO_4$

Next, multihole Qube 384 experiments were conducted to assess differences in hNa$_v$1.5 pharmacology between CaF_2 and $BaSO_4$. Potency IC_{50} values for a range of Na^+ channel inhibitors were comparable against hNa$_v$1.5 between the different seal enhancers (Figure 2).

A



B

CHO-hNa$_v$1.5 Multihole experiments	IC_{50} (µM)	
	3 mM Ca^{2+} + 120 mM F^-	5 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

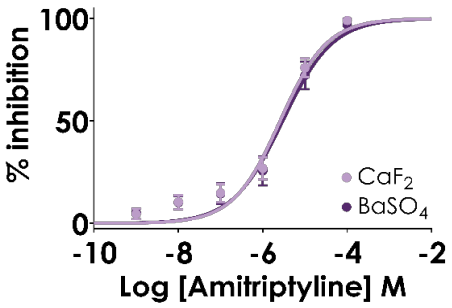


Figure 2. CaF_2 versus $BaSO_4$ – hNa$_v$1.5 pharmacology. A) Representative sweep plots (left) and current-time (I-t) plots (right) for hNa$_v$1.5 inhibition by amitriptyline. There was no difference in cumulative inhibition of hNa$_v$1.5 by increasing concentrations of amitriptyline between CaF_2 and $BaSO_4$. **B)** Screening of a range of inhibitory compounds showed no difference in hNa$_v$1.5 pharmacology between CaF_2 and $BaSO_4$. Concentration-response curves for amitriptyline against hNa$_v$1.5 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 $BaSO_4$). CaF_2 and $BaSO_4$ data taken from two separate experiments.

$hCa_v1.2$ pharmacology: CaF_2 versus $BaSO_4$

In a similar manner, the effects of CaF_2 versus $BaSO_4$ on $hCa_v1.2$ pharmacology were assessed using a HEK293-$hCa_v1.2$ cell line and multihole QChips. Using $BaSO_4$ as the seal enhancer changes the kinetics of the channel (Figure 3). Ca^{2+} influx with CaF_2 activates calmodulin, which binds to the intracellular regions of the channel, facilitating Ca^{2+}-dependent inactivation[16-17]. Ba^{2+} is routinely used as a surrogate charge carrier in the study of Ca^{2+} channels[18]. As expected, Ca^{2+}-dependent inactivation of $hCa_v1.2$ was eliminated when Ba^{2+} was used as a surrogate carrier ion with $BaSO_4$ as the seal enhancer, also giving rise to larger $hCa_v1.2$ current amplitudes (mean ± S.D. – CaF_2: 0.79 ± 0.58 nA (N = 11) versus $BaSO_4$: 1.29 ± 0.89 nA (N = 56); Welch's *t*-test: $t_{(20)} = 2.36$; $p < .05$). Despite this, $hCa_v1.2$ pharmacology assessed using two inhibitory compounds, nifedipine and verapamil, was unaffected (Figure 4).

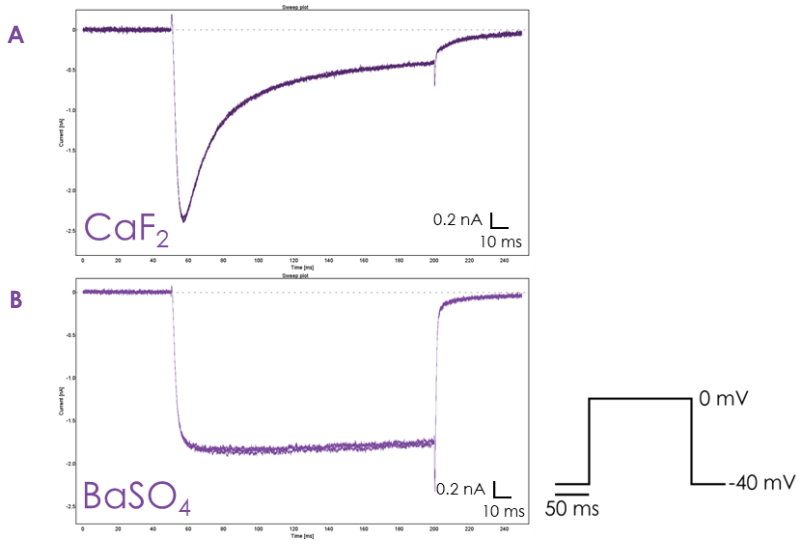


Figure 3. CaF_2 versus $BaSO_4$ – $hCa_v1.2$ kinetics. $hCa_v1.2$ exhibits Ca^{2+}-dependent inactivation when CaF_2 is used as the seal enhancer **(A)**. $BaSO_4$ as the seal enhancer (using Ba^{2+} as a surrogate carrier ion) confers loss of the Ca^{2+}-dependent inactivation of $hCa_v1.2$ observed with CaF_2 **(B)**. Example sweep plots derived from Sophion Analyzer v9.0.42.

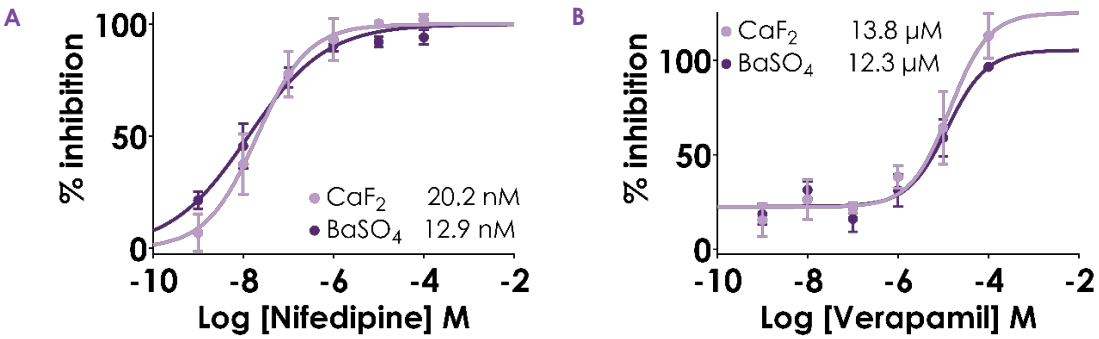


Figure 4. CaF_2 versus $BaSO_4$ – $hCa_v1.2$ pharmacology. Mean ± S.D. concentration-response curves for two common inhibitors against $hCa_v1.2$, nifedipine **(A)** and verapamil **(B)** (CaF_2: N = 2-5 wells per concentration; $BaSO_4$: N = 6-12 wells per concentration). Compound potencies (IC_{50} values) did not differ between CaF_2 and $BaSO_4$.

Seal stability following removal of seal enhancers

Exchange of solutions following gigaseal formation would provide flexibility to assay different ion channels using optimal recording solutions. However, stability of gigaseals formed in the presence of $BaSO_4$ (3, 5 or 10 mM extracellular Ba^{2+} and 50 or 70 mM intracellular SO_4^{2-}) were not maintained upon exchange for corresponding concentrations of extracellular Ca^{2+} (Figure 5), whilst those formed using CaF_2 as the seal enhancer (3, 5 or 10 mM extracellular Ca^{2+} and 120 mM intracellular F^-) were lost or greatly reduced following exchange for intracellular Cl^- across a range of concentrations (data not shown).

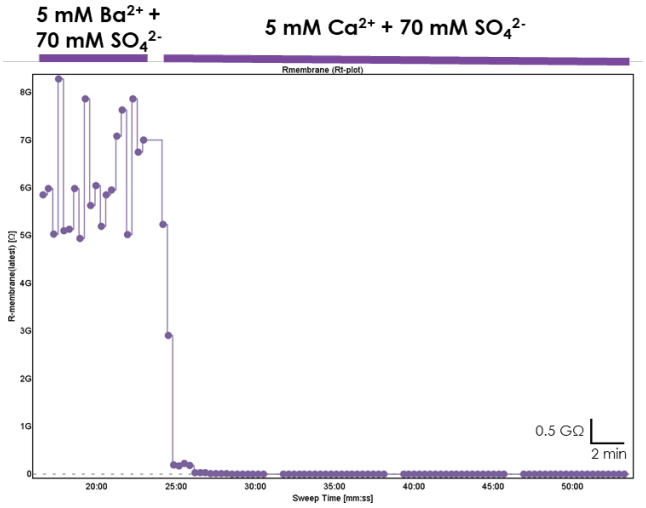


Figure 5. Ba^{2+}-Ca^{2+} solution exchange. Example $R_{membrane}$-time plot showing loss of a gigaohm seal formed using 5 mM extracellular Ba^{2+} and 70 mM intracellular SO_4^{2-} upon exchange for 5 mM extracellular Ca^{2+}.

Conclusion

In conclusion, although no correlation was found between salt K_{sp} values and gigaseal formation, $BaSO_4$ was identified as an equivalent seal enhancer to CaF_2 in planar patch clamp electrophysiology. $BaSO_4$ and CaF_2 were characterised across two different ion channels, $hNa_v1.5$ and $hCa_v1.2$. Whilst increasing concentrations of F^- caused depolarising shifts in the voltage dependence of inactivation of $hNa_v1.5$, SO_4^{2-} had no effects on $hNa_v1.5$ biophysical properties. Additionally, there was no difference in the pharmacological effects of inhibitory compounds against $hNa_v1.5$ or $hCa_v1.2$ between CaF_2 and $BaSO_4$ seal enhancers. $BaSO_4$ could be used as the seal enhancer when recording from non-K^+-conducting Ca^{2+}-activated channels, such as the Ca^{2+}-activated Cl^- channel TMEM16A, allowing more accurate estimation of the free intracellular Ca^{2+} concentration.

Methods

Experiments were conducted using a Sophion Bioscience Qube 384 with QChip 384 (single hole) and QChip 384X (multihole) consumables. Temperature was maintained at 22 °C using the Qube temperature control module.

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.

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References

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