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APPLICATION REPORT

Recordings of the electrogenic sodium-calcium exchanger (NCX) using novel low-noise Q-amp amplifiers on QPatch.

With the improved Signal-to-Noise Ratio (SNR) it is now possible to record electrogenic transporter currents with current windows down to 5 pA allowing recordings of non-cumulative concentration-response relationships and resulting IC_{50} values.

Introduction

Membrane transporters are integral membrane proteins that facilitate the movement of ions, small molecules and macromolecules across cell membranes. Transporters can be divided into two main classes, depending on whether they rely directly on ATP hydrolysis to move substrates against their concentration gradient (active transport), or whether they 1) rely on established electrochemical gradients (secondary active) or 2) facilitate diffusion across the membrane without the use of energy (passive transport):

- ATP-driven transporters (P-type, F-type or V-type and ATP-binding cassette (ABC) transporters) ***(active transport)***
- Solute-carrier (SLC) transporters ***(secondary active or passive transport)***

The SLC transporters bind specific solutes and undergo conformational changes to move them across the membrane. Depending on their mechanism they can be further categorized into:

- **Uniporter:** Move a single type of molecule down its concentration gradient
- **Symporters (co-transporters):** Move two or more different molecules/ions in the same direction, using the electrochemical gradient of one to drive the other against its gradient
- **Antiporters (exchangers):** Move two or more different molecules/ions in opposite directions.

The Na^+/Ca^{2+} exchangers (NCXs) are electrogenic antiporters expressed in heart, kidney, brain and skeletal muscle where they contribute to Ca^{2+} homeostasis by buffering the intracellular [Ca^{2+}], driven by the electrochemical gradient of the substrates, primarily Na^+ (figure 1). It typically works by transporting one Ca^{2+} out of the cell in response to three Na^+ ions entering, but under depolarized conditions it can reverse its operation (reverse mode). The most widely expressed isoform is NCX1, which is of interest as a target for cardiac safety, due to its involvement in maintaining the plateau phase of the cardiac action potential through its forward action[1].

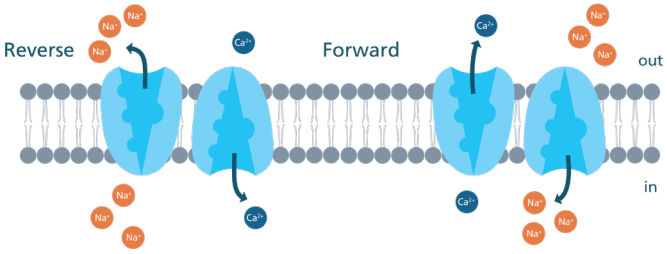


***Figure 1**: Simplified representation of the ion transport modes by NCX. In the forward mode (right) NCX transfers three Na^+ ions into the cell down their electrochemical gradient, which drives the transport of one Ca^{2+} ion from the cytosol to the extracellular environment. The ions are transported in the opposite direction when NCX is operating in the reverse mode (left). Adapted from Scognamiglio et al. 2025[2].*

As transporters undergo significant conformational changes to bind and move solutes across the membrane, they are typically acting slower (10^2 – 10^4 molecules/s) than ion-channels (10^7 – 10^8 ions/s). Resultingly, transporter currents are smaller and inherently more difficult to assess using traditional electrophysiology techniques, such as patch clamp.

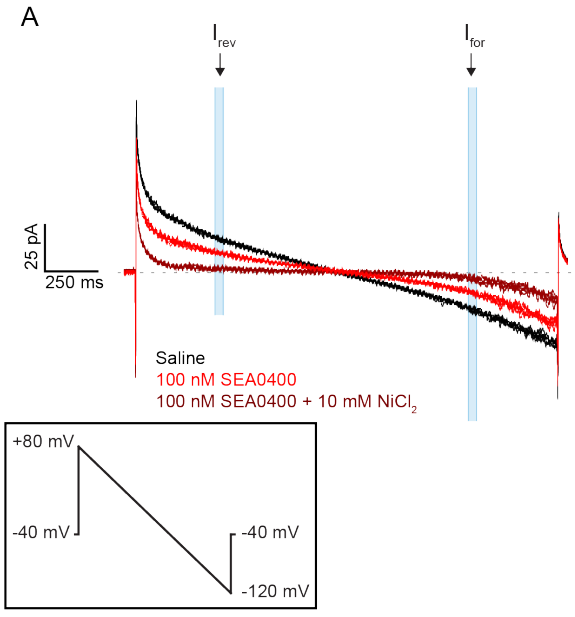
In this report we demonstrate how our new Q-amp amplifier with up to 3-fold lower Root Mean Square (RMS) noise compared to our previous[1] amplifier, together with optimized intra- and extra-cellular solutions to minimize background currents, allows us to record NCX current windows (NCX1 and NCX2) down to 5 pA, with a resolution that permits us to record non-cumulative compound concentration-response plots and IC_{50} values.

Results and discussion

NCX current recordings

The NCX current was recorded in both the reverse (I_{rev}) and forward (I_{for}) direction in response to a voltage ramp protocol (see methods). In accordance with literature, the NCX current is defined as the Ni^{2+} - sensitive current. To avoid background currents from other ion channels the experiment was run at constant osmolarity and Cl^- concentration and in the presence of blockers of K_v, Na_v and Na^+/K^+ - ATPase (see methods for solution details). A representative NCX2 current is displayed in figure 2.

The Ni^{2+} sensitive NCX1 and NCX2 currents were recorded in parallel in a population of non-induced cells and a population of cells exposed to 24 h induction. The resulting Ni^{2+} sensitive currents in the reverse and forward directions are plotted in figure 3.



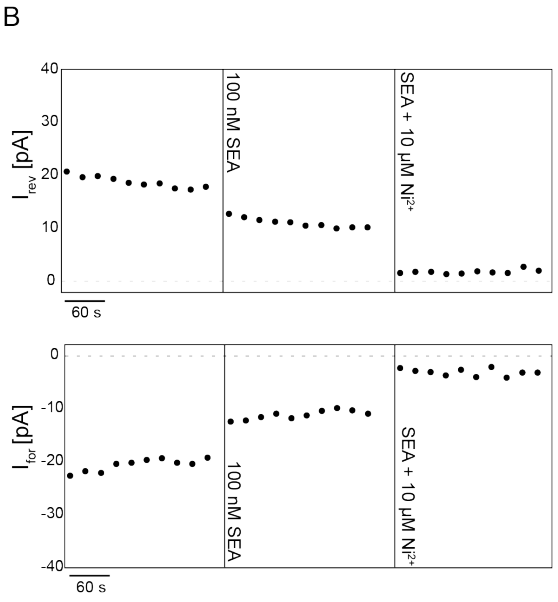


Figure 2: A) *Representative NCX2 current in response to a voltage ramp protocol (insert), before (black), after addition of 100 nM SEA0400 (red) and 10 mM $NiCl_2$ (hammerdose, dark red). The blue cursors indicate the voltages at which the reverse (I_{rev}, +40 mV) and forward (I_{for}, -80 mV) are calculated.*

B) *Median I_{rev} (top) and I_{for} (bottom), recorded every 20 seconds, as a function of time. The vertical lines indicate addition of 100 nM SEA0400 followed by addition of 10 mM $NiCl_2$.*

[1] Existing instruments can be upgraded

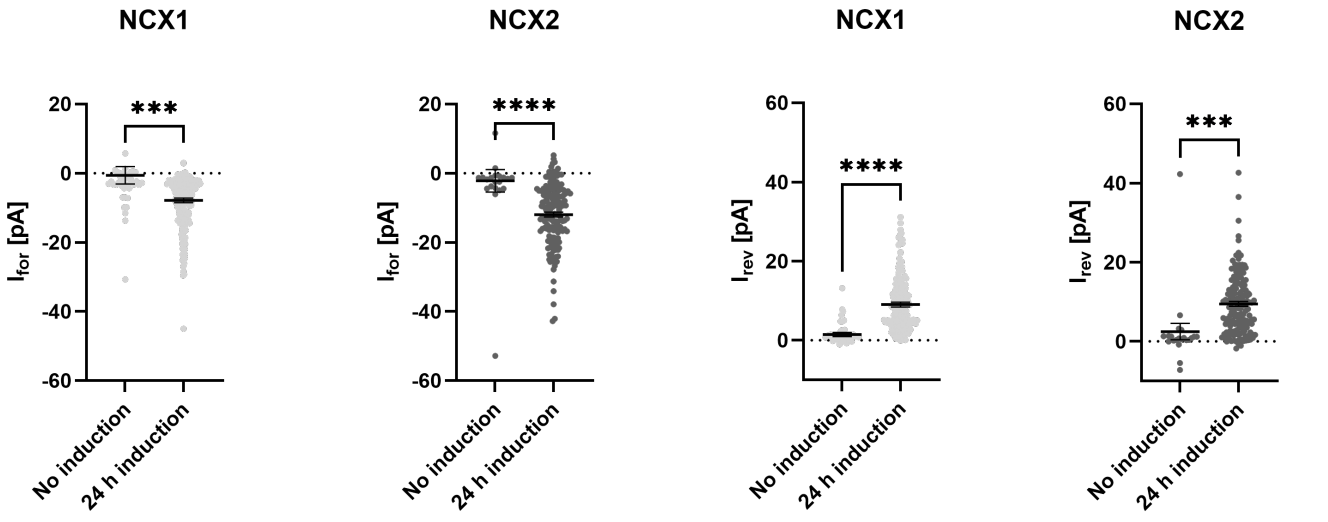


Figure 3: *The Ni^{2+} sensitive NCX current was recorded in the reverse (I_{rev}) and forward (I_{for}) direction in NCX1 (light grey) and NCX2 (dark grey) expressing cells upon induction for 24 hours, together with control cells that were not induced. The difference in sensitive currents between non-induced controls and induced cells were evaluated with an unpaired t-test and the significance is indicated by the number of stars with (***) indicating P < 0.001 and (****) indicating P < 0.0001.*

The mean sensitive currents recorded are listed in table 1:

	No induction		24 h induction	
	I_{rev} [pA]	I_{for} [pA]	I_{rev} [pA]	I_{for} [pA]
NCX1	1.4 ± 0.5	-1 ± 3	9.0 ± 0.6	-7.9 ± 0.7
NCX2	3 ± 2	-2 ± 3	9.5 ± 0.6	-12.0 ± 0.7

Table 1: *The Ni^{2+} sensitive NCX1 and NCX2 currents in the reverse (I_{rev}) and forward (I_{for}) direction, recorded in a population of non-induced cells and populations of cells exposed to 24 h induction. Values are mean ± SEM.*

Assay success rates

To evaluate assay success rates, the data was filtered according to the following filter settings:

- QC filters: cell resistance R_{mem} > 500 MΩ; cell capacitance C_{slow} > 4 pA; leak current $|I_{leak}|$ < 25 pA
- NCX current filters: I_{rev} > 5 pA; I_{for} < -5 pA

The assay success rates are plotted in figure 4 and the mean currents, upon application of the filters above are listed in Table 2.

We obtain WC success rates > 80% and NCX current detection rates of 32% and 39%, for NCX1 and NCX2, respectively.

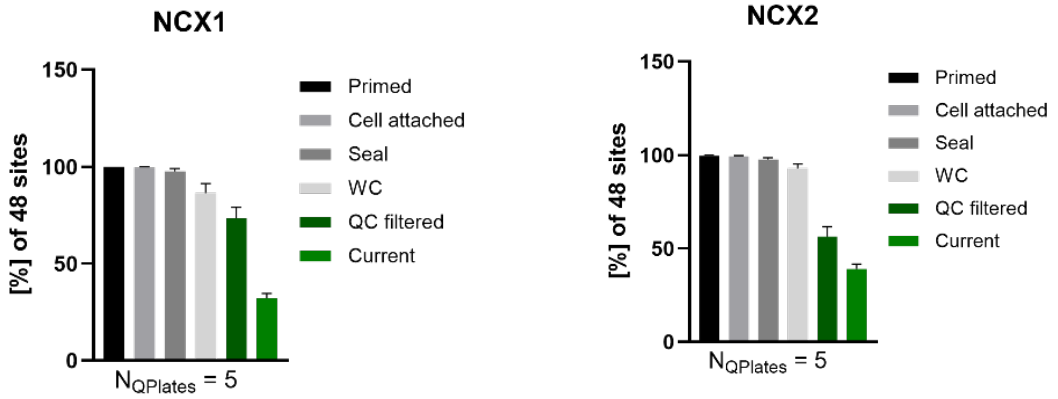


Figure 4: *Assay success rates in populations of induced NCX1 (left) and NCX2 (right) cells.*

	Mean current	
	I_{rev} [pA]	I_{for} [pA]
NCX1	17 ± 4	-14 ± 2
NCX2	13 ± 1	-16 ± 2

Table 2: The Ni^{2+} sensitive NCX1 and NCX2 currents in the reverse (I_{rev}) and forward (I_{for}) direction, after application of QC and current filters (listed above). The data is mean ± SD of the current extracted from 10 QPlates (NCX1) or 6 QPlates (NCX2).

Test of tool compounds

The following two common NCX blockers were tested:

- ORM10103 (5 pt conc-response from 10 µM)
- SEA0400 (5 pt conc-response from 10 µM)

As the NCX currents are low and very sensitive to rundown we did non-cumulative (1 dose per cell) concentration-response plots (see figure 5) and the extracted IC_{50} values are listed in table 3. The data was normalized according to:

Norm NCX block = (Input – Baseline)/
(Full response – Baseline)

The current filters utilized for the concentration-response plots were the following:

- NCX current filters: I_{rev} > 10 pA; I_{for} < -10 pA

A minimum of 2 QPlates were required to obtain 1 compound IC_{50} with 3 data-points per concentration.

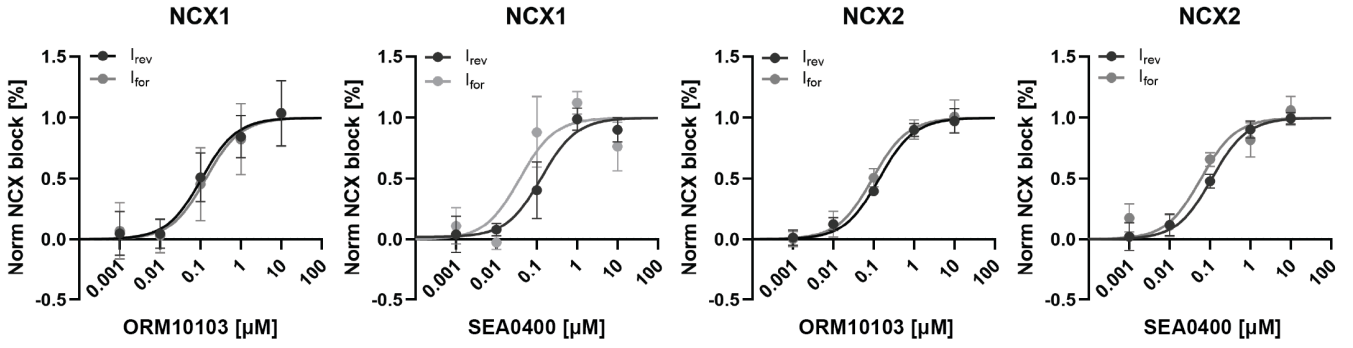


Figure 5: *Normalized NCX1 and NCX2 block concentration-response plots recorded in the reverse (I_{rev}, dark grey) and forward (I_{for}, light grey) in response to ORM10103 (5 pt concentration-response from 10 µM) (left) and SEA0400 (5 pt concentration-response from 10 µM).*

Transporter	Tool compound	IC_{50} (normalized block)		Literature values	
		I_{rev} IC_{50} [nM]	I_{for} IC_{50} [nM]	I_{rev} IC_{50} [nM]	I_{for} IC_{50} [nM]
NCX1	ORM10103	110 ± 20	140 ± 30	960[3]	780[3]
NCX2		140 ± 20	94 ± 6	N/A	N/A
NCX1	SEA0400	130 ± 50	40 ± 30	28 ± 5[4], 40[5]	111 ± 43[5]
NCX2		110 ± 10	60 ± 20	980 ± 50[4]	N/A

Table 3: *Tool compound, ORM10103 (top rows) and SEA0400 (bottom rows), IC_{50} values in nM recorded in the reverse (I_{rev}) and forward (I_{for}) direction, listed for NCX1 and NCX2, together with literature values where available (right).*

Materials and methods

Compound preparation

ORM10103 was dissolved in DMSO (10 mM stock) and resuspended in the experiment solution including 0.05% pluronic F-127. End solutions including controls contained 0.001% DMSO and 0.05% pluronic F-127.

SEA0400 was dissolved in DMSO (10 mM stock) and resuspended in the experiment solution. End solutions including controls contained 0.001% DMSO.

Cell lines

The source of the cell lines is confidential.

Cell lines tested:

- CHO-Flip-In-T-REx-human NCX1
- CHO-Flip-In-T-REx-human NCX2

Induction:

The Flip-In-T-REx cell lines were induced using 1 µg/mL doxycycline 24 h prior to experiment.

Solutions

IC base (in mM): 20 NaCl; 10 $CaCl_2$; 10 EGTA; 10 HEPES; 100 CsCl (pH = 7.2, ~ 293 mOsm)

EC base (in mM): 2 $CaCl_2$; 1 $MgCl_2$; 10 HEPES; 4 KCl; 115 NaCl; 10 Glucose (pH = 7.4, ~ 250 mOsm, [Cl^-] = 125 mM)

Exp solutions (prepared on the exp day):

EC Saline (in mM): EC base **+ 35 CsCl** (~320 mosm, [Cl^-] = 160 mM)

EC Blockers (in mM): EC base **+ 35 CsCl; 100 nM TTX; 50 µM Ouabain** (~320 mosm, [Cl^-] = 160 mM)

EC Blockers + $NiCl_2$ (in mM): EC base **+ 15 CsCl; 10 $NiCl_2$; 100 nM TTX; 50 µM Ouabain; 10 mM sucrose** (~320 mosm, [Cl^-] = 160 mM)

IC (in mM): IC base + 4 Na_2ATP (pH 7.2 with CsOH, ~300 mOsm)

Voltage protocol

NCX ramp protocol (figure 6):

Clamping between protocols: -40 mV

Time between protocol start: 20 s

Filtering: optimized for low noise

Sampling frequency: 2000 Hz

Cut-off frequency: 100 Hz

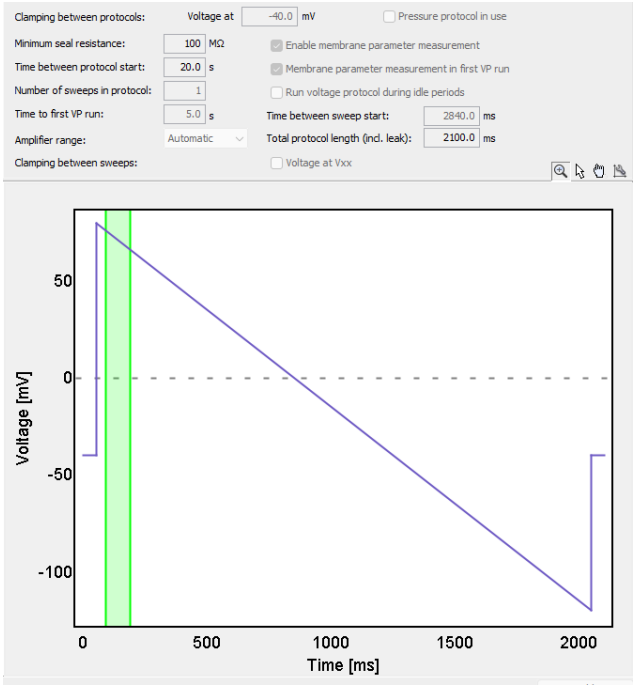


Figure 6: *NCX ramp protocol exported from analyzer.*

Application protocol

The experiment timeline:

LP1 EC Saline – 3 VP runs
LP2 EC Blockers – 30 VP runs - MTP pos 1
LP3 EC Blockers + Comp – 10 VP runs - MTP pos 2
LP4 EC Blockers + $NiCl_2$ – 10 VP runs - MTP pos 3

An example of the 8x(4x3) MTP layout for a compound-response experiment:

	1	2	3	4	5	6
A	EC + Na blockers 0µM	EC + Na blockers 0µM	EC + Na blockers + NiCl2 0µM	EC + Na blockers 0µM	ORM10103 0.0100µM	ORM10103 + NiCl2 0.0100µM
B	EC + Na blockers 0µM	ORM10103 0.00100µM	ORM10103 + NiCl2 0.00100µM	EC + Na blockers 0µM	ORM10103 0.100µM	ORM10103 + NiCl2 0.100µM
C	EC + Na blockers 0µM	ORM10103 0.0100µM	ORM10103 + NiCl2 0.0100µM	EC + Na blockers 0µM	ORM10103 1.00µM	ORM10103 + NiCl2 1.00µM
D	EC + Na blockers 0µM	ORM10103 0.100µM	ORM10103 + NiCl2 0.100µM	EC + Na blockers 0µM	ORM10103 10.0µM	ORM10103 + NiCl2 10.0µM
E	EC + Na blockers 0µM	ORM10103 1.00µM	ORM10103 + NiCl2 1.00µM			
F	EC + Na blockers 0µM	ORM10103 10.0µM	ORM10103 + NiCl2 10.0µM			
G	EC + Na blockers 0µM	EC + Na blockers 0µM	EC + Na blockers + NiCl2 0µM			
H	EC + Na blockers 0µM	ORM10103 0.00100µM	ORM10103 + NiCl2 0.00100µM			

References

1. N Jost *et al.* ORM-10103, a novel specific inhibitor of the Na+/Ca2+ exchanger, decreases early and delayed afterdepolarizations in the canine heart. *British Journal of Pharmacology* vol. 170 768–778 Preprint at https://doi.org/10.1111/bph.12299 (2013).
2. Scognamiglio, A. *et al.* Druggability of Sodium Calcium Exchanger (NCX): Challenges and Recent Development. *International Journal of Molecular Sciences* vol. 26 Preprint at https://doi.org/10.3390/ijms26188888 (2025).
3. N Jost *et al.* ORM-10103, a novel specific inhibitor of the Na+/Ca2+ exchanger, decreases early and delayed afterdepolarizations in the canine heart. *British Journal of Pharmacology* vol. 170 768–778 Preprint at https://doi.org/10.1111/bph.12228 (2013).
4. Iwamoto, T. *et al.* Molecular Determinants of Na+/Ca2+ Exchange (NCX1) Inhibition by SEA0400. *Journal of Biological Chemistry* **279**, 7544–7553 (2004).
5. Nagy, N. *et al.* Selective Na +/ Ca 2+ exchanger inhibition prevents Ca 2+ overload-induced triggered arrhythmias. *Br. J. Pharmacol.* **171**, 5665–5681 (2014).

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