Drug Effects on human Na^+/Ca^{2+} Exchanger and Implications for Drug Development.

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Introduction

Background.

- The Na^+/Ca^{2+}-exchanger (NCX) plays an important role in cellular calcium homeostasis under physiological but also pathological conditions.
- The pharmacological potential and risks of NCX have been debated for many years. The consequences of changes in activity and mode of operation of NCX are still discussed. Mostly a potential beneficial effect of NCX reverse mode inhibition under ischemic conditions has been suggested.
- There is ongoing effort to find specific and direction-specific NCX inhibitors, but inadvertent NCX inhibition is usually not tested during drug development.

Objectives.

- Investigate and compare NCX pharmacology in iPSC derived cardiomyocytes and in overexpressing HEK293 cells using different assay types with medium to high throughput.
- Develop a novel 96-well based electrophysiological assay to investigate compound effects on NCX. Validate the assay and test performance with a showcase screening using 28 compounds with different cardiac risk profile.

Literature.

- Amran et al., Cardiovascular Drug Reviews, Vol. 21, No. 4, pp. 255–276
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Methods

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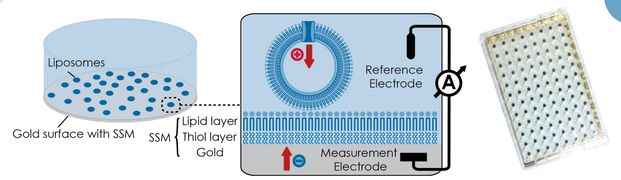
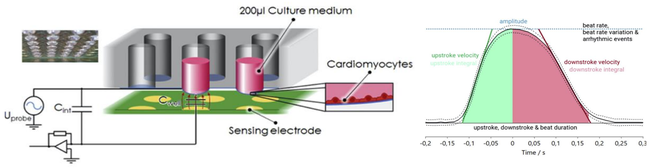


Solid Supported Membrane (SSM)-based electrophysiology (SURFE²R N1 and SURFE²R 96SE)

is a sensor-based method, which allows the resolution of low amplitude electric events in biological membranes, bringing the advantages of electrophysiology to the membrane transporter field. A large capacitive sensor coated with a high amount of membrane vesicles or proteoliposomes is used to detect changes in electrical potential that are generated by electrogenic transport processes with high amplification. The method is applied in mechanistic and pharmacological transporter research.



FLEXcyte 96 Technology

is an impedance-based technology, which allows recordings from human iPSC derived cardiomyocytes to be performed on a pro-maturation flexible substrate. Characterization of electric and mechanical properties of the beating cells allows the assessment of efficacy and safety of new drug candidates on a human model system.

Development of an electrophysiological screening assay

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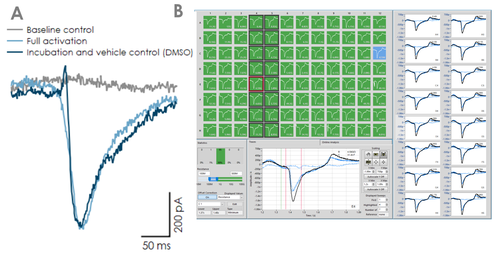
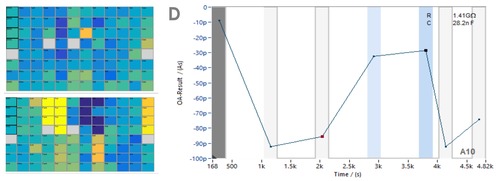
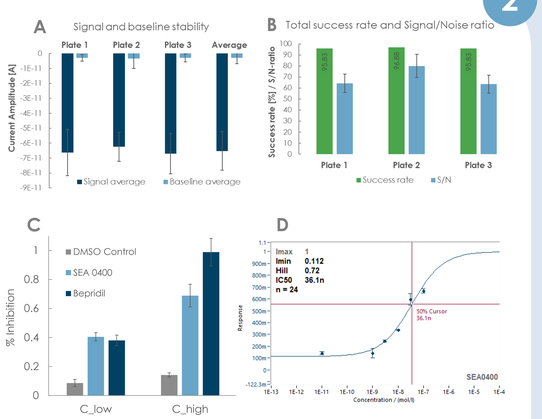


NCX was overexpressed in HEK293 cells, the plasma membrane was purified and applied to a SSM sensor.

Cell line was provided by AXXAM S.p.A.. NCX activity was triggered by application of Ca^{2+} to Na^+ loaded membrane vesicles on the sensor. Currents can be triggered repetitively with a stable amplitude. Nanion's SURFE²R 96SE platform was used for application and recording. A: Individual NCX current response in a single well. B: Overview of a full 96 well plate in the instrument software. C: Heatmap view of a 96 well sensor displaying the maximum current amplitude before (top) and after (bottom) addition of different compounds. D: Current amplitude maximum in an individual well over time: Baseline, maximum current (2x), inhibitor (2x), wash out (2x).





Performance of the assay was validated by means of plate-to-plate reproducibility, the success rate and SEA 0400 pharmacology.

A: Amplitudes of the full activation were homogenously distributed and reproducible. Wells with very low amplitude were not used for analysis. B: The success rate was high (>95%), and the signal-to noise ratio stable and sufficient. C+D: Applying the activation sequence introduced in the right panel, it is possible to analyze compound effects as single point (C) or dose-dependent manner (D). Applying these two modes we determined either 2 IC_{50}s (n=8) per plate or 11 compounds in two concentrations (n=4). DMSO vehicle control showed a stable base-line and the current was inhibited in the expected concentration range by SEA 0400.

NCX in human iPSC derived cardiomyocytes

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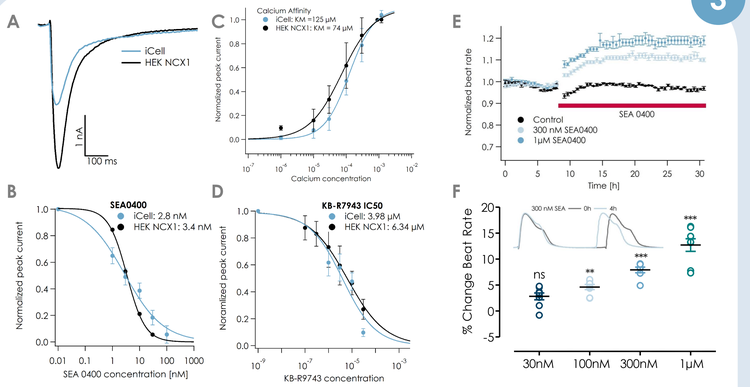


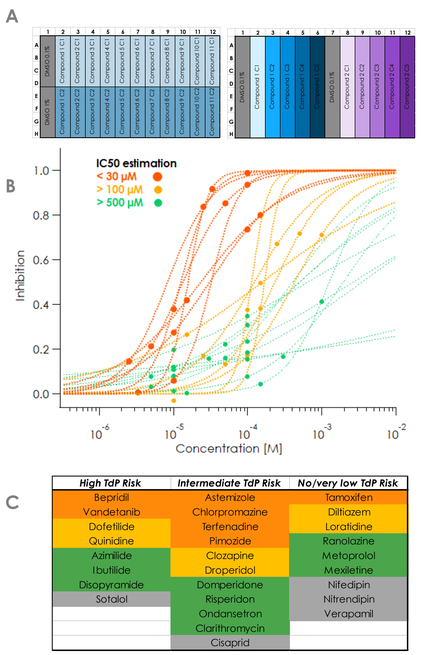
iPSC derived CMs have large NCX specific currents - revealed by SSM-based electrophysiology.

SSM-based electrophysiology (SURFE²R N1) was applied to detect NCX activity in human iPSC-CMs. The current characteristics and pharmacology was compared to HEK 293 cell expressing NCX1 (A-D). We measured significant and specific NCX activity, indicating a high expression level of NCX in these cells. This functional evidence for high NCX activity in a human model system points to the relevance of inadvertent and intended pharmacological effects on NCX in the human heart.

Investigating physiological effects of NCX inhibitors using FLEXcyte 96 technology.

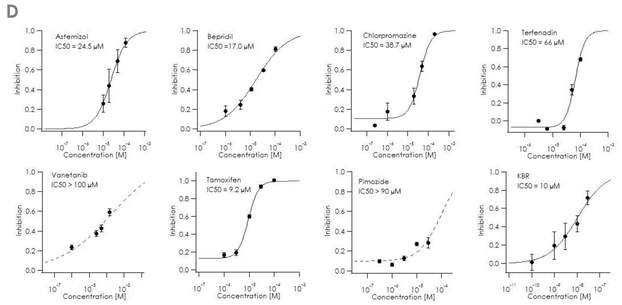
hiPSC-CMs were seeded on the FLEXcyte 96 well plate and the beat rate was determined via impedance recordings. Addition of SEA0400 significantly increased the beat rate in a dose dependent manner as a long-term effect (E+F). Although reproducible, this finding is contradictive to published research, therefore further evaluation is necessary for a comprehensive interpretation.

Showcase screening of cardiac drugs



C

High TdP Risk	Intermediate TdP Risk	No/very low TdP Risk
Bepridil	Astemizole	Tamoxifen
Vandetanib	Chlorpromazine	Diltiazem
Dofetilide	Terfenadine	Loratidine
Quinidine	Pimozide	Ranolazine
Azimilide	Clozapine	Metoprolol
Ibutilide	Droperidol	Mexiletine
Disopyramide	Domperidone	Nifedipin
Sotalol	Risperidon	Nitrendipin
	Ondansetron	Verapamil
	Clarithromycin	
	Cisaprid	



28 drugs categorized as high, intermediate, and low proarrhythmic risk were tested – several showed inhibiting effects on NCX currents.

The drugs were picked and separated into risk categories by the CIPA initiative. We chose these compounds because of their cardiac context and good availability. The list contained bepridil, which is known as unspecific NCX inhibitor. Initially each compound was tested at a low and a high concentration, depending on solubility (A left). B: Several of the drugs did have an effect on NCX activity, tough usually only at high concentrations. Fife compounds caused a disturbance of the measurement (marked grey). IC_{50}'s were estimated using the two concentration datapoints and assuming that a full inhibition can be accomplished. The drugs were ranked by the estimated IC_{50}'s. There was no connection between the risk categories and the effect on NCX (C). In a second step the drugs that showed activity plus the NCX inhibitor KBR as control were further characterizes by a more detailed IC_{50} estimation applying a different addition scheme (A right). For most of the drugs the estimated IC_{50} was confirmed.

Assessment of impact

This small screen was performed as a default test scenario and the data must be interpreted with care. The validity of the results is supported by the reliable detection of known NCX inhibitors. However, whether the observed inhibition effects are NCX specific, in particular at high concentrations, should be evaluated using complementary techniques. Physiological implications are unlikely given the high concentrations - unless accumulation effects occur, as described e.g. for Bepridil.

Conclusions

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- SSM-based electrophysiology is a suitable tool to investigate NCX currents in iPSC-CMs and can also be upscaled to a format that allows screening compound effects with reasonable throughput. Unlike in other screening assays, the NCX directly and isolated from secondary effects.
- Our findings demonstrate the presence of functional NCX in hiPSC-CMs which until now has been poorly investigated. This emphasizes the relevance of cardiac NCX pharmacology.
- Applying the FLEXcyte 96 technology to investigate CM beating behavior we found a suitable tool to observe physiological effects of NCX inhibition.
- From a set of compounds with cardiac activity, a surprising 25% had a significant effect on NCX activity in the low micromolar range. Although these experiments must be refined in order to be able to make assumptions about the physiological implications, this is an intriguing observation hinting to a potential role of NCX in drug safety.

→At this point we can state that we could put some perspective on a possible role of NCX in safety pharmacology. We outlined the relevance for further investigations of the electrogenic transporter and demonstrated that novel technologies can help break new ground also in well established research areas.