Abstract

The Sodium-Calciun Exchangers (NCX) play an important role in the cellular calcium homeostasis under physiological and pathological conditions. NCX has been of interest as a pharmacological target for many years, in particular because clinical trials involving inhibitors of the sodium-proton exchanger, NHE, have delivered mixed results. Inhibition of the reversed mode of NCX is thought to be beneficial in ischemia/reperfusion injury by reducing cardiac, neuronal and renal ischemia areas. Moreover, inhibition of NCX has been proposed to exhibit an antiarrhythmic effect and therefore, may provide a novel target for the treatment of a variety of arrhythmogenic pathologies. So far, a number of studies have shown promising results but investigations are limited by the currently available NCX inhibitors such as KB-87943, SEA-040 and 3HI-6 which are only partially specific.

To drive the progress in pharmacological NCX research, we provide a method which allows the resolution of low amplitude electrochemical events in biological membranes, bringing the advantage of electrophysiology to the transporter field. It enables real-time activity measurement of electrogenic SLC-transporters, K+ -transporters and ion pumps, based on the application of a lipid membrane in intracellular or bacellular membranes. Unlike cell-based electrophysiology, a large capacitive sensor coated with a high amount of membrane vesicles or proteoliposomes is used. In this way any process that generates an electrical potential can be regulated with high amplitude. The method was established in the 90's and has only recently been scaled up to a 96-well format.

Development of a screening tool for NCX

To screen compounds for an effect on NCX for safety purposes or during development of novel inhibitors, new methods to measure NCX function are needed. At the current time, functional measurement of NCX range from patch-clamp, calcium flux assays, Langendorff-perfused hearts to studies in whole animals. We have developed an electrophysiological method to investigate NCX function which is based on the solid supported membrane (SSM) technology. SSM is a versatile gain for expressing NCX or human induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) were used in a single-well or a 96-well SSM electrophysiology device and NCX was recorded from these cells. NCX was activated using Co2+ in the bath and inhibited by Co2+ and other compounds.

Measuring NCX activity in human iPSC derived cardiomyocytes

Human iPSC derived cardiomyocytes are being investigated as a model for cardiac safety assessment. To measure native NCX in these cardiomyocytes a cell based assay was developed. Cardiomyocytes were detached from the culture dish and added to the lipid coated SSM sensor. Where the cell connected with the lipid layer. Upon mixing of the sensor with high fluidic speed, the cells detached again, but a sheet of the cell membrane remains on the sensor. NCX currents were then measured in these sheets. For a higher NCX signal female cardiomyocytes were used. This method enables the efficient investigation of the isolated cardiac NCX current in a native membrane.

Summary and Future prospects

We have developed two novel methodological approaches for the functional investigation of NCX and have recorded NCX from HEK cells and iPSC-CMs. NCX was activated by Ca2+ in the buffer and could be inhibited by a number of pharmacological agents.

Future Experiments

• Further validation and optimization of the method is required.
• We intend to develop a third complementary method using a Helipat-like system to investigate the influence of NCX inhibitors or cross reactions of NCX inhibitors and pro-arrhythmogenic drugs on the beating behavior of iPSC derived cardiomyocytes.

• The developed methods shall be applied in a project to characterize drug effects on NCX in more detail.
• The next step intended is to screen the proarrhythmogenic drugs of the CPA initiative and use the high throughput method for effects on NCX.

Contact us if you are interested in collaborating with us to:
• Apply the high throughput method for the development of novel NCX inhibitors.
• Evaluate the use for NCX safety testing of new drug candidates using the here presented technological method.

Label-free analysis of Na+/Ca2+-exchanger (NCX) isolated from iPSC-derived cardiomyocytes

Maria Barthmes, Andre Bazzone, Ulrich Thomas, Andrea Brüggemann, Michael George, Niels Fertig, Alisson Oberbrugger

Nanion Technologies GmbH, Ganghoferstr. 70A, 80339 Munich, Germany, contact: Info@nanion.de

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