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Validation of Na+, K+ -ATPase Isoform Endogenous to Cardiomyocytes for High Throughput Rb Uptake Assay Using Cor.At® Cardiomyocytes & ICR8000™

Introduction

Human Na+,K+-ATPase is the target for cardioglycosides such as digitoxin and digoxin which are used in treatment of congestive heart failure and related conditions; thus, it is emerging as an important drug target1. The Na+, K+ pump generates electrochemical gradients that are used to drive the coupled transport of many ions and nutrients across the plasma membrane as it actively exports three Na+ ions with the concomitant import of two K+ ions, hydrolyzing one ATP molecule in the process. In non-cell-based assays, the activity of Na+,K+-ATPase has been determined by using purified enzyme preparations to hydrolyze ATP2. In cell-based assays, techniques such as patch clamping, fluorescence, H3-Ouabain binding, and radio-tracer (Rubidium86), and cold Rubidium flux assay have been used in either recombinant cell lines or in cells other than primary cardiomyocytes3-5. However, primary cardiomyocytes cannot be used as they lack the homogeneity, sensitivity and surface binding properties critical to developing cell based assays in a HTS format6. In view of the availability of standardized pure cardiac myocytes with functional expression of all essential cardiac ion channels, Aurora Biomed validated Na+,K+-ATPase in cultured Cor.At® cardiomyocytes derived from transgenic mouse embryonic stem cells.

Materials & Methods

Rb+ uptake by cultured Cor.At® cardiomyocytes was carried as follows:

1. Culture and maintenance of Cor.At® cardiomyocytes: The cells endogenously expressing Na+,K+-ATPase were provided frozen in 96 well MTPs, thawed and cultured as per instructions provided by supplier of the cells. On the 4th day of culture, Rb+ uptake experiment was carried out.

2. Rb+ Uptake: Cells were washed once with 200 uL of Rb+ Uptake Buffer and Rb+ uptake was initiated by applying 200 uL of Rb+ Uptake Buffer.

a. The Rb+ uptake profile: Rb+ uptake activity of the cells was studied by incubating the cells at specific time intervals in presence of Aurora Biomed's Rb+ Uptake Buffer.

b. Dose response: The cells were incubated in the Rb+ Uptake Buffer containing appropriate dose of test compound. It was followed by incubation for 15 minutes at room temperature (~22 °C).

3. Wash: Residual Rb+ and compound were removed by four successive washes with 200 uL of SPA-Wash Buffer.

4. Cell Lysis: Intracellular samples were obtained by whole cell lysis with the application of 200 uL Lysis Solution.

5. Analysis: The level of Rb+ in the intracellular samples was measured by Aurora Biomed's ICR8000™ using flame atomic absorption spectroscopy.

Results

1. Ready to use cells: The Cor.At® cardiomyocytes were observed to be ready for use for the assay after 3 to 4 days of maintenance.

2. Expression of Na+, K+-ATPase isoform: The Rb+ uptake profile observed in the assay indicated expression of the this isoform in these cardiomyocytes (Figure 1) showing maximum uptake of Rb+ in about 80 minutes, with an exponential uptake seen from 0 to 50 minutes. Thus these cells can be employed for 15 minutes of incubation in a HTS-format compound screening assay.

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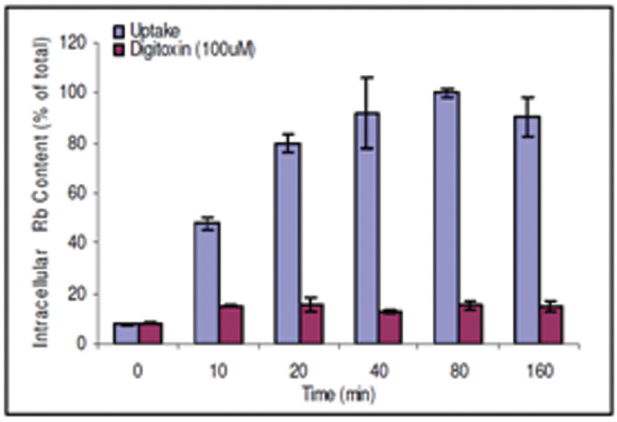

Figure 1. Rb+ uptake profile of Cor.At® cardiomyocytes ATPase isoforms.

3. Potency of standard blocker of Na+, K+-ATPase:

A complete blockage of Na+,K+-ATPase isoform was observed with 100 µM of digitoxin. Digitoxin was determined to have an IC50 value of 6.82 µM (Figure 2). The potency of digitoxin also displays a similar relation between mouse and Chinese hamster Na+,K+-ATPase isoforms5.

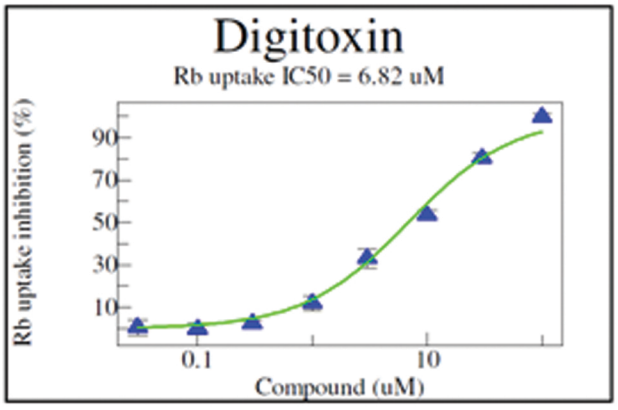

Figure 2. Cor.At® cardiomyocytes endogenously expressing Na+, K+ -ATPase isoform displaying IC50 value for digitoxin.

Conclusion

The data suggest that the Cor.At® cardiomyocytes can be used as a predictive model for identification of modulators of Na+, K+-ATPase in its original cardiac environment with Rb uptake assay and ICR8000™.

References

1. De Munari, S. et al. (2003). Structure-based design and synthesis of novel potent Na+,K+- ATPase inhibitors derived from a 5 alpha, 14 alphaandrostane scaffold as positive inotropic compounds. J Med Chem 46 (17): 3644-3654.
2. Zahler, R. et al. (1997). Sodium kinetics of Na,K-ATPase alpha isoforms in intact transfected HeLa cells. J Gen Physiol 110 (2): 201-213.
3. Chen, W. and W. Wu (2002). The asymmetric, rectifier-like I-V curve of the Na/K pump transient currents in frog skeletal muscle fibers. Bioelectrochemistry 56 (1-2): 199-202.
4. Geibel, S. et al. (2003). Conformational dynamics of the Na+/K+-ATPase probed by voltage clamp fluorometry. Proc Natl Acad Sci U S A 100 (3):964-969.
5. Gill S, Gill R, Wicks D, Despotovski S & Liang D (2004). Development of an HTS assay for Na+, K+-ATPase using non-radioactive rubidium ion uptake. Assay Drug Dev Technol 2(5):535-42.
6. Automated patch clamp of Cor.At® Cardiomyocytes in the PatchXpress 7000A (MDS-Analytical Technologies) System.Brochure. AxioGeneis AG.

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