I. Abstract

GABA developmental switch controls the intracellular chloride concentration and shifts the functional role of GABA between neonatal and adult stages from excitation to inhibition. The switch is mediated by the change in expression of two major ion co-transporters Na+/K+2Cl- co-transporter (NKCC1) and K+/Cl- co-transporter (KCC2). Alterations in this mechanism may result in CNS disorders such as autism spectrum disorder, epilepsy, and schizophrenia. Ion flux detection is the common approach to measure electro-neutral CCCs activity. While several approaches are available such as radioactive flux and fluorescence based techniques including Thallium and Fluorescence in situ hybridization (FISH), Rb+ flux assay was found to be the most optimal due to its specificity for measurement of the co-transporter activity. Here we describe the development of a high-throughput rubidium uptake assay for measuring NKCC1 function, and the HTS campaign of 1.2 million compounds using Rb+ uptake detection by AAS technology (ICR12000™, Aurora Biomed, Vancouver, Canada) to identify modulators of NKCC1.

II. Introduction

Development of hyperpolarizing effect of GABA requires a decrease of intracellular [Cl-] to a change in the ratio of two ion transporters with different stoichiometry in the same direction. The balance of excitation and inhibition, which is a central hypothesis for the etiology of autism, was found that NKCC1 influx flux a role in seizures observed in autistic children and seizure frequency in epilepsy patients. NKCC1 co-transport Na+, K+ and Cl- at 1 : 1 : 2 stoichiometry. It’s a non-electronic transporter. Manual and automated electrophysiological methods for measuring chloride transport activity could not be practical due to the normal range of excitation and inhibition, which is a central hypothesis for the etiology of autism. Thallium and FISH have been used to visualize NKCC1 expression and activity in the GABAergic neurons. Recent studies have shown that Rb+ flux assay is a robust assay for high throughput screening of novel NKCC1 modulators.

III. Objectives

- Develop & optimize an HTS assay for NKCC1 drug target
- Screen compound libraries against NKCC1 target
- A focused library of 1450 compounds was screened in a high throughput campaign using the Rb+ influx assay by AAS technology. Rb+ uptake detection by AAS technology was found to be the most optimal assay for measuring NKCC1 function, and the HTS campaign of 1.2 million compounds using Rb+ uptake detection by AAS technology (ICR12000™, Aurora Biomed, Vancouver, Canada) to identify modulators of NKCC1.

IV. Results & Discussion

Cell Line Generation: Insoluble NKCC1 cell line was generated by Life Technologies and validated by functional test (T1+ uptake)

Expression test (CHO, HEK) & Functional test (T1+ uptake) (A) NKCC1 transfected CHO-K1, HEK or 293T. (B) NKCC1 transfected CHO-K1, HEK or 293T.

Assay Development (5 wells): Rb+ uptake detection by AAS was developed by Aurora Biomed. Readings were compared between both AAS and ICR12000™. The window of detection determined by activated (2 min) and basal influx providing supportive evidence to its sensitivity, error bars represent standard error of n=46.

Dose response curve of bumetanide on NKCC1 Rb+ influx activated for 2 min (A). Positive control 30uM of bumetanide (A), and negative controls as activated Rb+ influx in the absence (B) and presence of 1U of digenase (C).

Optimized liquid handling by increasing sample volume improves readout:

Test Compound | AAS-I2000 (Bom) | ICR12000™ (Bom) | Mix 1uM (Bom) | Same rank for all 3 assays
---|---|---|---|---
Bumetanide | 0.36 | 0.37 | 0.35 | 2
Remacemide | 0.74 | 1.6 | 0.3 | 2
Remacemide | 0.36 | 0.37 | 0.35 | 2

V. Conclusion

The Rb+ flux assay is a robust assay for high throughput screening of novel NKCC1 modulators. Specific NKCC1 inhibitors were screened in a high throughput campaign using the Rb+ flux assay.

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