ION TRANSPORTER

VALIDATION OF A ROBUST LABEL FREE SCREENING ASSAY FOR NKCC1 COTRANSPORTER

Background

The cotransporter NKCC1 (encoded by SLC12a2) is a membrane protein which mediates the tightly coupled movement of Na^+, K^+, and Cl^- ions across the plasma membrane of many cell types (e.g. brain, heart) with major roles in regulation of cellular volume and intracellular ion concentrations[1]. NKCC1 is gaining increased attention as a potential therapeutic target for treating multiple CNS disorders as dysregulation of neuronal chloride homeostasis may be associated with neurological disorders such as epilepsy, chronic neuropathic pain, and autism[2]. Because 2 Cl^- ions are translocated across the membrane along with 1 Na^+ ion and 1 K^+ ion, NKCC1 cotransport is electrically neutral. The non-radioactive rubidium (Rb^+) flux assay is a method of choice to study non electrogenic pumps, including NKCC1[3]. Data shown below report the concentration-dependent effects of bumetanide, a selective NKCC1 inhibitor in the presence and in the absence of ouabain, a Na/K ATPase inhibitor. Data were generated from HEK cells transiently expressing NKCC1, using the Ion channel Reader (ICR8100) from Aurora Biomed™.

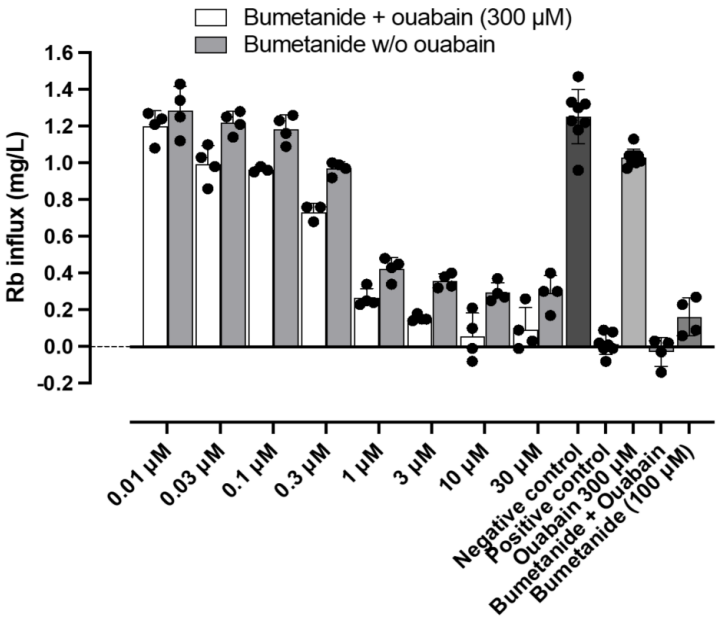

Figure 1) Representative rubidium influx results obtained from a 96-well plate run on the Ion Channel Reader series (ICR8100). Rubidium influx was normalized to the maximum (negative control: rubidium loading in the absence of compounds) and minimum response (positive control: No rubidium load).

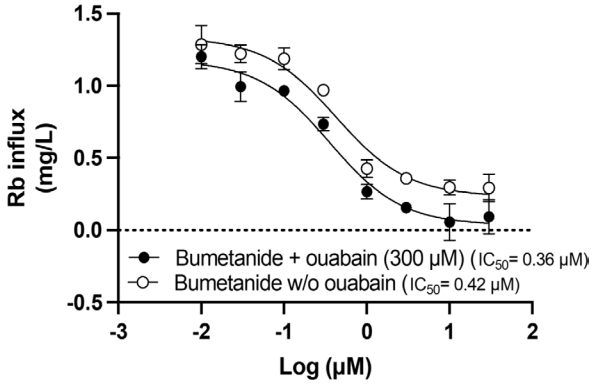

Figure 2) Dose response relationships for bumetanide and bumetanide with ouabain (300 µM). Eight concentrations (0.01 to 30 µM) model assuming a slope factor (Hill coefficient, nH) equal to 1. Data are shown as mean ± SD.

Conclusion

Bumetanide inhibits rubidium influx in NKCC1 expressing cells with an IC_{50} of 0.36-0.42 µM.

In the absence of ouabain, the highest concentration of bumetanide (100 µM) inhibited about 80% of the rubidium influx, indicating that NKCC1 is the main contributor to rubidium influx into the cells. 300 µM ouabain, combined with 100 µM bumetanide fully inhibited rubidium influx, indicating that Na/K ATPase's contribution to the rubidium intake is about 20%. These experiments also suggest that no other mechanisms contribute to the rubidium influx measured in those cells.

Using the rubidium flux assay, Aurora Biomed Inc. can determine the concentration-dependent effects of test compounds on the human NKCC1 pump expressed in mammalian cells.

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Instruments utilized

ICR8100

ICR12000

Specifications	ICR 8100	ICR 12000
Throughput	up to 5000 wells/day	up to 60000 well/day
Minimum Sample Volume	50 uL	20 uL
Sensitivity	0.05pp m dection limit	0.05pp m dection limit
Precision	<5% CV	<5% CV

References

1. Pedersen SF et al. Physiology and pathophysiology of Na^+/H^+ exchange and Na^+-K^+-2Cl- cotransport in the heart, brain and blood. Am J Physiol Regul Integr Comp Physiol 291: R1-R25, 2006.
2. Ben-Ari, Y. NKCC1 Chloride Importer Antagonists Attenuate Many Neurological and Psychiatric Disorders. Trends Neurosci. 2017, 40, 536–554.
3. Gill S et al. A high-throughput screening assay for NKCC1 cotransporter using nonradioactive rubidium flux technology. Assay Drug Dev Technol 2017; 15(4) 167-177.