Cell-based Rubidium Flux Assay for HTS of Kv1.3 Channels

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I. Abstract

Kv1.3, a voltage-gated K^+ channel expressed by human T lymphocytes is widely recognized as a potentially valuable therapeutic target. Some peptides, like agitoxin and margatoxin in scorpion venom, and some non-peptide small molecules are known to inhibit this channel. However, there are limitations on the effectiveness of these compounds and the pharmaceutical industry has put much emphasis on the discovery and development of new Kv1.3 blockers. The major limiting factor for such a development though has been the lack of HTS technology needed to screen large compound libraries. Aurora Biomed has developed a cell-based assay in 96-well format to facilitate high-throughput screening of Kv1.3. This assay uses rubidium as a tracer for K^+ and is then analyzed by Aurora Biomed's Ion Channel Reader (ICR). The assay provided a Z' factor of 0.86 and a more than 6-fold window of detection. The two blockers agitoxin and margatoxin resulted an IC_{50} values of 0.6 and 2 nM, respectively which are about 3 fold higher than those determined from patch clamp.

II. Introduction

The voltage-gated Kv1.3 channel and calcium-activated K^+ channel IKCa1 are important potassium channels expressed by human T lymphocytes. High expression of Kv1.3 channels in T lymphocytes has been implicated in the pathogenesis of multiple sclerosis-like inflammation in animal models, as the blockade of these channels leads to a reduction in inflammation. Because of such observations, Kv1.3 has become an attractive *therapeutic target* for immunotherapy[1,2]. The search for Kv1.3 blockers or molecules able to prevent channel expression could, therefore, provide novel anti-inflammatory treatments.

Although some peptide and non peptide blockers of this channel have been isolated from scorpion venoms and sea anemones, such molecules have limitations for therapeutic use. Moreover, the pharmaceutical industry's search for effective blockers of Kv1.3 is hampered by the lack of high throughput screening (HTS) tools for Kv1.3 channel modulation, which can provide greater potential than current technologies, including patch clamp, radiolabeled Rb^+ flux and ligand binding.

The present studies describe the development of an high-throughput cell-based assay to screen compounds for Kv1.3 channel modulation.

III. Materials & Methods

A. Electrophysiology

Electrophysiology experiments were conducted using standard whole cell patch clamp techniques. The bath solution contained (in mM) 0.90 CaCl2, 2.67 KCl, 1.47 KHPO4, 0.50 MgCl2, 138 NaCl, and 8.10 Na2HPO4. The pipette solution contained (in mM) 140 KCl, 1 MgCl2, 1 EGTA, and 20 HEPES.

For plotting I-V curves, cells were held at -80mV, and then stepped to a depolarizing voltage for 1s to record the peak current.

B. Efflux assay

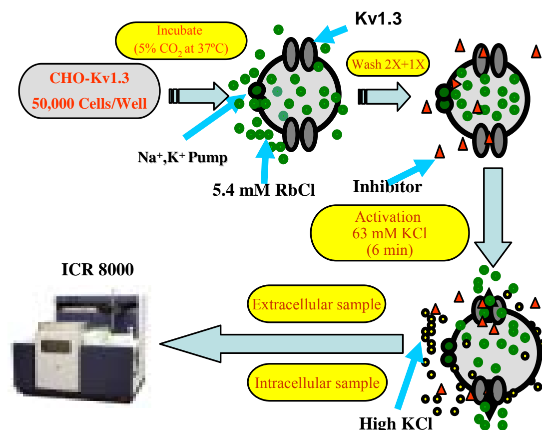

Figure 1. Assay steps include seeding of cells in multiwell plate, incubation, loading of cells with Rb^+, washing of extracellular Rb^+, activation of Kv1.3 channels with high K^+, aspiration of extracellular (efflux) Rb^+ sample, preparation of intracellular (lysate) Rb^+ sample and analysis on ICR series for Rb^+ content in the samples.

IV. Results

A. What was the I-V response of the CHO-Kv1.3 cells in whole cell patch clamp studies?

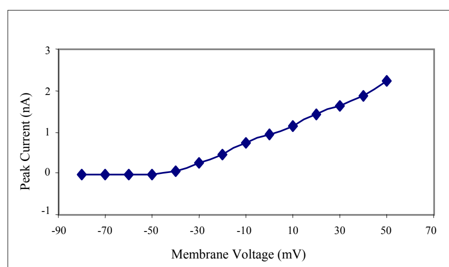

Figure 2. I-V response of the CHO-Kv1.3 cell line showing continuous rise in channel current in response to membrane potential from -50 to +50 mV.

B. What was the potency of a selective blocker of Kv1.3 in whole cell patch clamp studies?

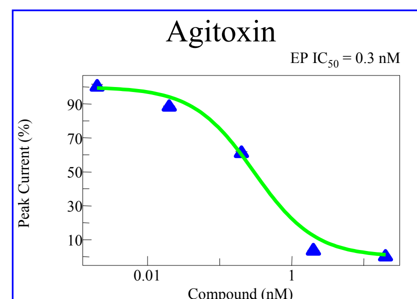

Figure 3. Agitoxin, a selective blocker of Kv1.3, generated an IC_{50} value of 0.03 nM as determined by patch clamp.

C. Did Kv1.3 channel respond to changes in membrane potential in flux studies; with KCl activation? What was the window of detection?

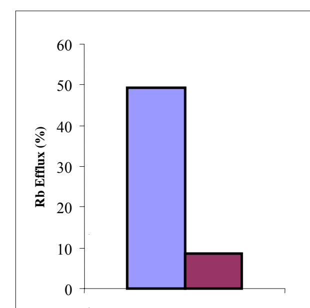

Figure 4. The channel responded to rise in membrane potential effected by extracellular KCl concentration. After 6 minute activation time using 33mM KCl, the maximal activation-induced efflux (blue bar) was 49.3% in comparison to basal efflux (red bar) of 8.5%. The activated efflux was 6X higher than basal efflux corresponding to an appreciable window of detection. The assay generated a Z' factor value of 0.86 that corresponds to an excellent assay.

D. What was the potency of agitoxin on Kv1.3 channels using cold Rb^+ flux assay?

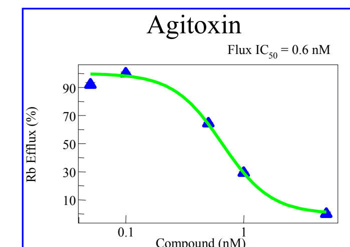

Figure 5. Curve-fits and flux IC_{50} of agitoxin on CHO Kv1.3. An IC_{50} value of 0.6nM was determined by Aurora Biomed's rubidium flux assay.

E. What was the potency of another blocker, margatoxin, on Kv1.3 channels using cold Rb^+ flux assay?

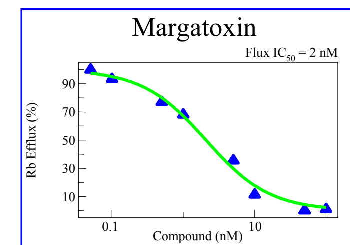

Figure 6. Curve-fits and flux IC_{50} of margatoxin on CHO Kv1.3 channel. An IC_{50} value of 2 nM was determined by rubidium flux assay.

V. Conclusion

The Rb^+ flux assay can be used to accurately identify compounds that modulate ion flow through Kv1.3 channels. Precise high-throughput screening can be accomplished using this flux assay coupled with Aurora Biomed's Ion Channel Reader Series. The robustness and sensitivity of the assay was reflected by a very high Z' factor value (0.86).

VI. Acknowledgements

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VII. References

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