HEK-Nav1.5 Cell Line

Human Nav1.5 and Cardiac Function

Human Nav1.5 (SCN5A) ion channel also known as hH1a is an important cardiac channel involved in safety pharmacology. Mutations in the cardiac sodium gene SCN5a, have been found to contribute to inherited cardiac arrhythmias, Brugada syndrome and long QT (LQT3) syndrome[1]. Furthermore, abnormal heart rhythms due to LQT3 are more likely to be fatal than those due to types LQT1 (KVLQT1) or LQT2 (hERG).

It has recently come to light that non-specific binding of drugs with the cardiac Nav1.5 channel can alter normal cardiac rhythm. For having a predicted role in cardiac safety screening, Nav1.5 has been included in cardiotoxicity screening panel.

Therefore, HEK-Nav1.5 cell line is an important cell line in the panel of targets used in assessing the cardiac safety profile of new chemical entities.

Our HEK-Nav1.5 cell line

- **Stably expresses Nav1.5 channel**
- **Validated using electrophysiology and flux assay**
- **Suitable for high-throughput screening**

Validation

The cell line has been validated using traditional patch-clamp whole cell recordings as well as cold-lithium flux assay in a high throughput format to investigate Nav1.5 channel activity.

Electrophysiology

Electrophysiology experiments were conducted using whole cell standard patch clamp technique. The bath solution contained (in mM) 0.90 $CaCl_2$, 2.67 KCl, 1.47 $KHPO_4$, 0.50 $MgCl_2$, 138 NaCl, and 8.10 Na_2HPO_4. The pipette solution contained (in mM) 140 KCl, 1 $MgCl_2$, 1 EGTA, and 20 HEPES.

For I-V plots, cells were held at -70mV, stepped to -120mV for 20ms, and then stepped to a depolarizing voltage for 20ms to record the peak current. For IC_{50} experiments, an I-V plot was carried out in the presence of a test compound (Fig. 1).

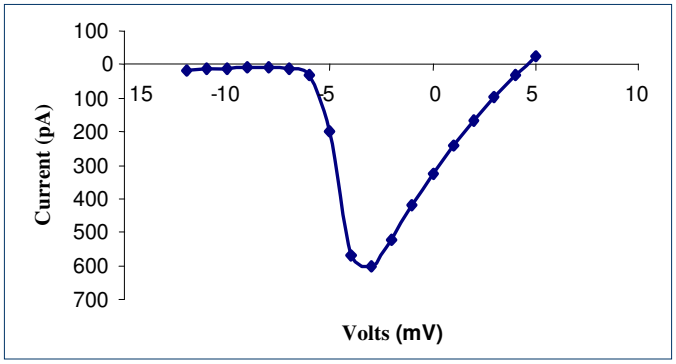


Fig. 1. I-V plot of Nav1.5 currents

The potencies of two known Nav1.5 blockers, TTC, and procainamide have been determined using patch clamp (Fig. 2). All recordings were done in duplicate with 5 - 8 concentrations.

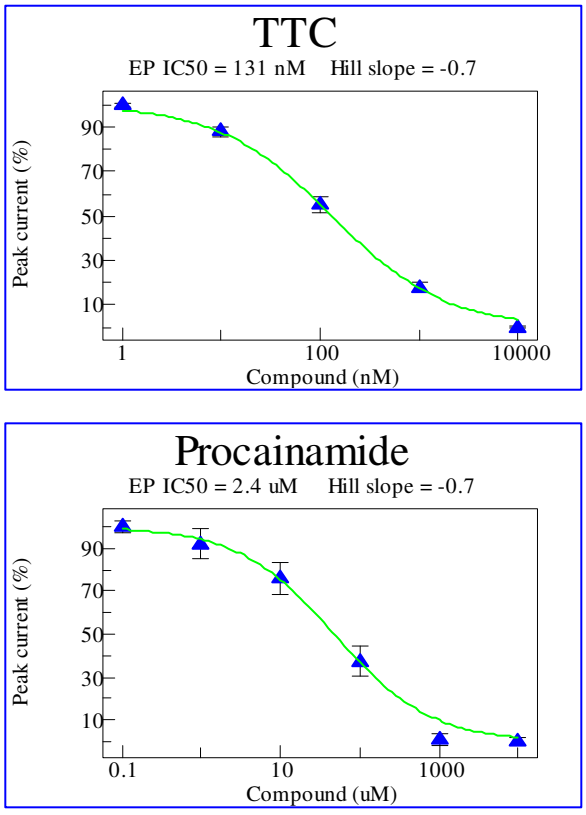


Fig. 2. Typical IC_{50} curves obtained using patch-clamp electrophysiology for Nav1.5 blockers.

1001 E. Pender St. Vancouver BC Canada V6A 1W2 Tel: 604.215.8700 Fax: 604.215.9700 www.aurorabiomed.com e-mail:info@aurorabiomed.com

Flux assay

Validation of the cell line was also carried using the Li^+ flux assay as follows:

Aurora Biomed's Ion Channel Reader Series (ICR) couples cold ionic flux assays with atomic absorption spectroscopy to provide a fully automated high throughput format for efficient screening of ion channels. Tracer ions not normally present in biological systems are utilized to help minimize background noise of the assay. For sodium channel applications, lithium is used as the tracer ions (Fig. 3)[3].
Using the Li^+ flux assay, the potencies of three known Nav1.5 blockers, TTC, and Amitriptyline have been determined (Fig. 4). All assays are performed in duplicate/triplicate with 8 concentrations.

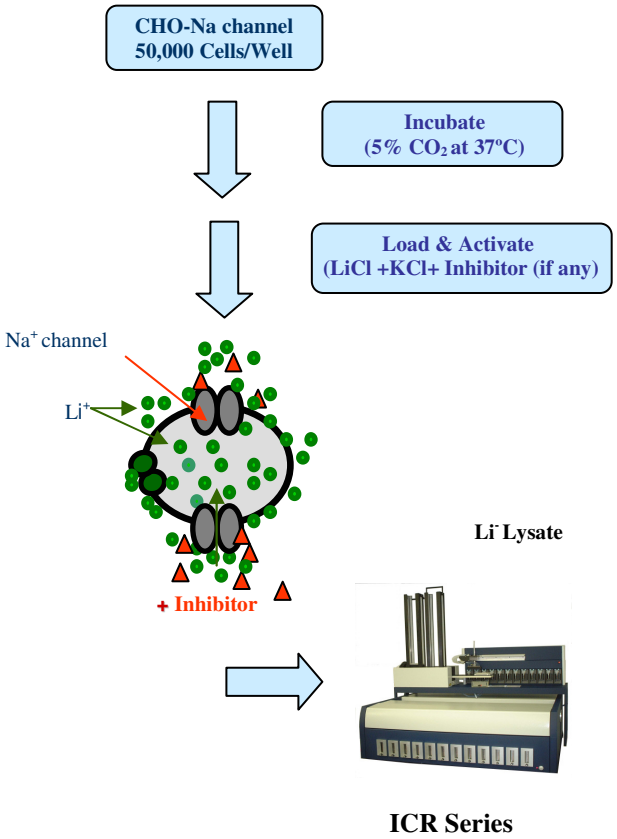
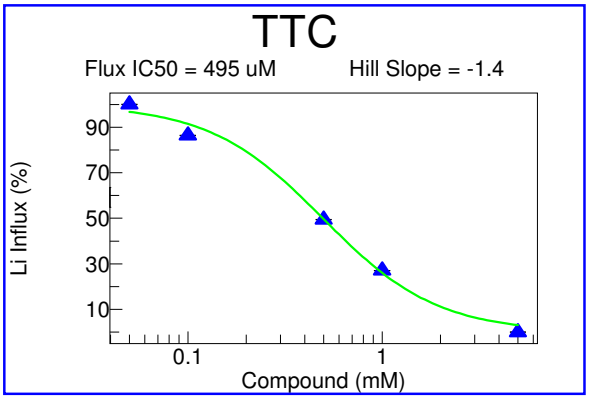


Fig. 3. Aurora Biomed's Li^+ Flux Assay



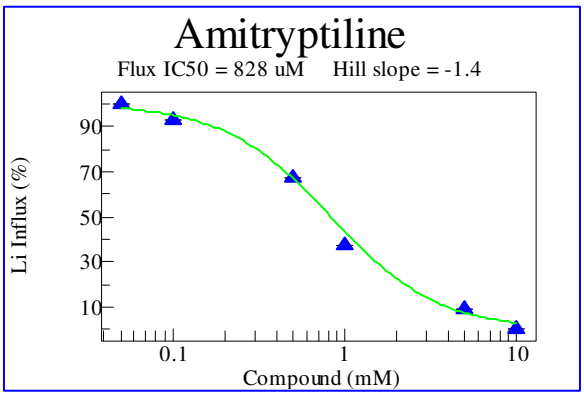


Fig 3. Typical IC_{50} curves obtained using Li^+ flux assay with the ICR.

References:

1. Viskin S : Long QT syndromes and torsades de pointes. Lancet 1999 ;354 :1625-33
2. Brown A : Ion channels in drig safety testing and drug discovery. 2002; PPT
3. Gill S, Gill R, Lee SS, Hesketh JC, Fedida D, Rezazadeh S, Stankovich L, and Liang D: Flux assays in high throughput screening of ion channels in drug discovery. Assay and Drug Develoomment Technologies 2003; 1: 709-17.

For further enquiries on our screening services and cell line licensing, please contact Aurora Biomed at the information below.

1001 E. Pender St. Vancouver BC Canada V6A 1W2 Tel: 604.215.8700 Fax: 604.215.9700 www.aurorabiomed.com e-mail:info@aurorabiomed.com