FDSS7000EX

Cell-based fluorescent assay for selectivity evaluation between two Na^+ channels subtypes

Introduction

Sodium voltage gated ion channels (Nav 1.x) are expressed in central neurons. Certain subtypes have been implicated in different forms of epilepsy and as such, have been targets in the development of anti-epileptic drugs. Very few methods are available to study voltage gated ion channels in high throughput which has impaired drug discovery for epilepsy. We describe here a fluorescence method, scalable in high throughput, based on the use of SBFI dye (e.g. invitrogen) in the Hamamatsu FDSS6000 screening system.

Materials and Methods

In order to determine which cellular concentration is optimal for the assays, 5,000 and 10,000 cells/well are plated in 384 well plates and kept for 48h at 37 °C. After 48h, growth medium is removed and 40 µl of SBFI (7.5, 10 or 15 µM) are added. After incubation of 3h at 37 °C, SBFI is removed and cells are washed with 40 µl of buffer then 40 µl of buffer are added. Plates are then placed in the FDSS6000 heated at 37 °Cand a 2 additions protocol is run:

- Baseline is recorded for 10 sec before first addition
- Compounds are added (10 µl, 5x in buffer) and signal is recorded for 5 min
- Veratridine is added (10 µl, 30 µM in buffer) and signal is recorded for 10 min

The following settings are used to record signal in the FDSS6000: excitation wavelengths 340 nm and 380 nm, emission wavelength 540 nm, exposure time 1 sec. Since SBFI is a dual excitation dye, the results are expressed as a ratio of the two excitations (emission at 340 nm/emission at 380 nm).

Results

Determination of assay conditions: Three parameters were taken into account in order to determine the best assay conditions; number of cells per well, SBFI concentration and DMSO concentration. The optimum number of cells per well in 384 format is 10,000, as this is the number necessary to achieve confluence. The best Z' (0.7) was obtained with 15 µM of SBFI whereas lower concentrations of SBFI gave Z' around 0.2 or 0.3. Tests showed up to 0.1 % of DMSO and there was no influence on baseline or Veratridine's effect on cells. Above 0.1 % of DMSO, baseline increases and Veratridine's effect drops significantly. With these results, the following conditions were chosen to perform the assays: 10,000 cells per well, SBFI concentration 15 µM and DMSO concentration 0.1 %. It is important to note that the effect of compounds diluted in DMSO, leading to a final concentration superior to 0.1 % cannot be interpreted.

Effects of Veratridine on two Nav channel subtypes: Data in fig.1 shows that Veratridine has the same effect on both channels. In both cases, EC_{50} is about 10 µM as seen in published literature.

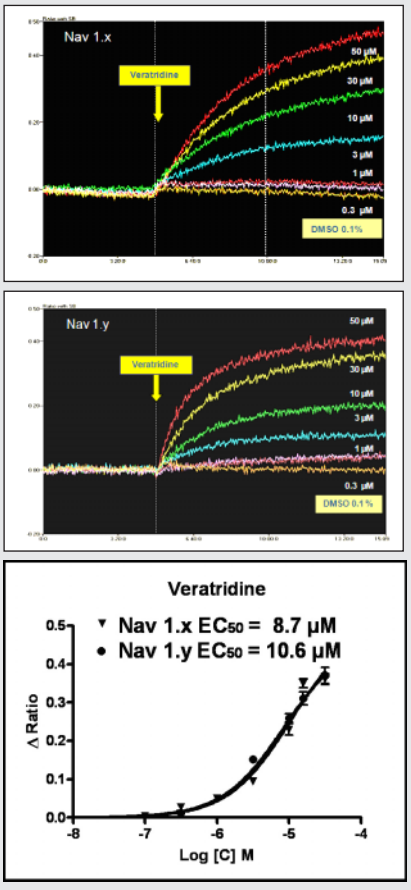


Fig. 1: Dose response curve of Vetratridine Nav1.X and Nav1.Y run on the Hamamatsu FDSS6000 with exposure times of 1 s.

Effect of antisodic compounds: the figure below shows the effect of reference antisodic compounds like Lidocain or Carbamazepine on Nav 1.x and 1.y sodium channels. In this assay, Phenytoin, Riluzole and TTX fully inhibit the effect of Veratridine. Carbamazepine displays only a slight effect on Veratridine response and the other compounds (Lidocain and Lamotrigine) show no effect. These compounds cannot be tested in higher concentrations because of the DMSO effect.

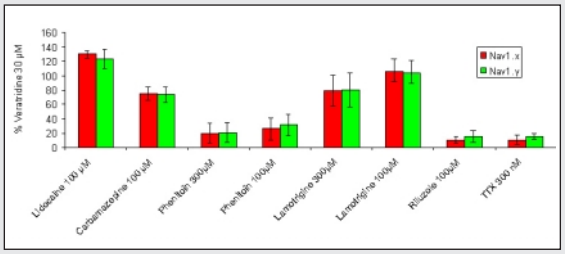
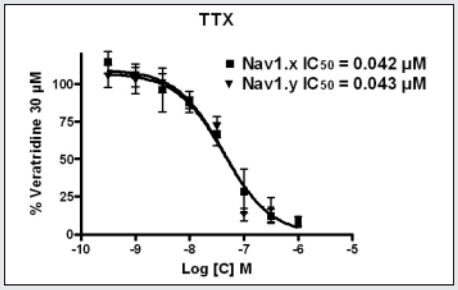


Fig. 2: Figure shows the effect of reference antisodic compounds like Lidocain or Carbamazepine on Nav 1.x and 1.y sodium channels.

Effect of reference compounds: Dose response curves were performed with reference compounds. TTX, Riluzole, Phenytoin and Fluoxetine block sodium channels in the same way and EC_{50} obtained are all in accordance with published literature.



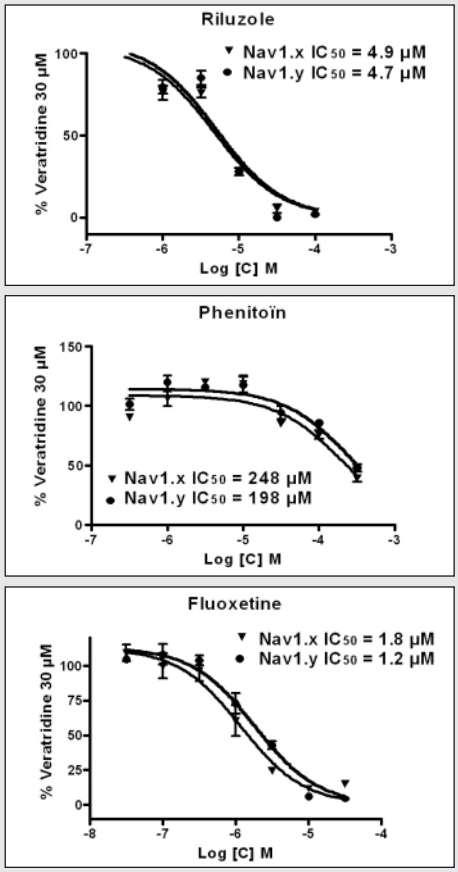


Fig. 3: Dose response curve of antagonist of Nav1.X and Nav1.Y run on Hamamatsu FDSS6000 with exposure times of 1 s.

Summary

The aim of the study was to evaluate SBFI dye as a potential screening method for voltage gated sodium channels. Results obtained by fluorescence in this study show a good correlation with electrophysiological data. As often in screening, compound autofluorescence is a limit for testing. One unexpected limit is the DMSO concentration which can impair measurement and "hide" compound effects.

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Cat. No. SBIS0088E02
NOV/2013 IP
Created in Japan (PDF)