

I. Abstract

Li⁺ is known to enter cells through Na⁺ channels at comparable permeability during patch clamp studies. Such studies support the use of Li⁺ as a tracer for Na⁺ channels and receptors in flux assays where Li⁺ was analyzed with the atomic absorption spectrophotometry (AAS)-based Ion Channel Reader (ICR) 8000. In these assays, cells are able to maintain a physiological membrane potential when Li⁺ is used to replace Na⁺. Channel or receptor activation can be achieved with raising the extra-cellular KCl concentration or with specific agonists. Li⁺ influx can be blocked or activated by specific blockers and activators respectively. In the present study, patch clamp was used to investigate the effects of using Li⁺ as a surrogate ion for Na⁺ in the bath solution. A comparison of potencies (IC₅₀) of specific blockers of Na⁺ channels was carried out in the presence of Na⁺/Li⁺. In addition, a comparison between patch clamp and flux assay was also carried out.

II. Introduction

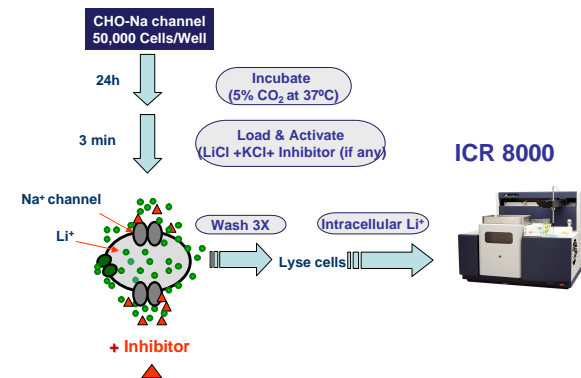
The activity of most ion channels can be studied by employing appropriate radioactive or nonradioactive tracers for example ²²Na and ¹⁴C-guanidinium for monitoring flux through sodium ion channels for pharmacological and toxicological studies¹. However, many HTS labs are reluctant to use the ²²Na - based radioactive flux assay format because of potential toxicity and health hazard associated with radioactivity.

Li⁺ is known to enter cells through Na⁺ channels² and the uptake of Li⁺ into the cells through voltage-dependent Na⁺ channels has been investigated for the pharmacological analysis of Li⁺ permeation. Therefore, Li⁺ has also been employed as an excellent tracer ion for Na⁺ channels and receptors due to their similarity in size and charge where Li⁺ has been analyzed by AAS-based ICR. An added advantage of using Li⁺ as a tracer is the fact that mammalian cells do not contain significant amounts of Li⁺ ions, thus providing a very low background noise in the assay. However, use of the tracer ion may effect the potencies of pharmacologically active compounds³.

To validate the use of Li⁺ as a tracer for Li⁺ flux assays, some known blockers of the cardiac sodium channel, SCN5A, were studied. Non-specific binding of drugs with SCN5A can alter normal cardiac rhythm. SCN5A channel modulation results using electrophysiology (EP) were compared to those using Li⁺ flux assay.

III. Materials & Methods

Li⁺ FLUX ASSAY



PATCH CLAMP

Electrophysiology was carried out using a PC2C patch-clamp amplifier. The patch clamp experiments were carried out with bath solution; NaCl (138 mM), KCl (5.4 mM), MgCl₂ (1 mM), CaCl₂ (1.8 mM) at pH 7.4 adjusted with NaOH. Borosilicate glass pipettes were filled with an internal solution containing KCl (140 mM), MgCl₂ (1 mM), EGTA (1 mM) and HEPES (20 mM) at pH 7.3 adjusted with KOH.

IV. Results

A. What is the I-V relation of investigated HEK/SCN5A cell line?

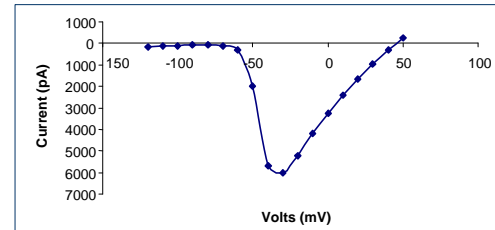


Figure 1. A mammalian (HEK) cell line stably expressing cardiac sodium channels showed robust current, fast channel kinetics and stable protein expression characterized by electrophysiology patch clamp techniques. The resting membrane potential is -60 mV and cells exhibit peak channel current at -40 mV. Thus the V_{rest} being close to I_{peak} suggest that the majority of channels are in the inactive state. In order to remove inactivation, cell needs to undergo hyperpolarization before being depolarized to open the channel.

B. Is Li⁺ detectable by ICR with good sensitivity?

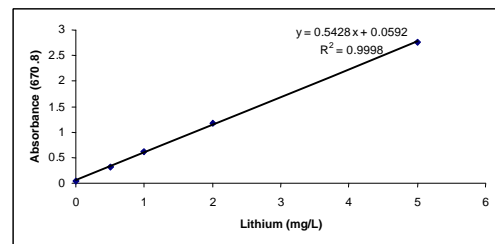


Fig. 2. ICR maintains the accuracy of detecting 0.02-5 mg/L concentrations of Li⁺ with r² of 0.9998, thus the ICR series is a powerful analytical tool for HTS of ion channels. The ICR series consists of the ICR 8000 and ICR 12000. The ICR 12000 has a 12- fold capacity of that of the ICR 8000, with a comparable sensitivity. A number of voltage- and ligand-gated channels can be studied using cold flux assay accompanied by the ICR Series.

C. Does the potency of TTX, an open state blocker of SCN5a channels differ in the presence of NaCl (138 mM) or LiCl (138 mM) in the bath solution?

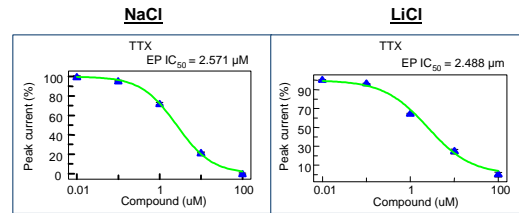


Figure 3. Potency of TTX (terodotoxin) did not significantly differ in the presence of NaCl (138 mM) or LiCl (138 mM) in bath solution.

D. Does the potency of TTC, a closed state blocker of SCN5a channel differs in the presence of NaCl (138 mM) or LiCl (138 mM) in bath solution?

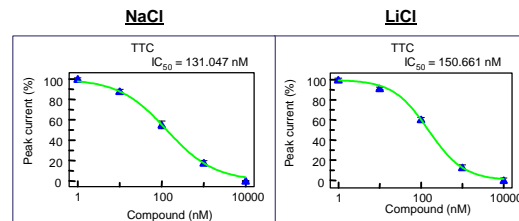


Figure 4. The IC₅₀ of TTC (tetracaine) also did not significantly differ in the presence of NaCl (138 mM) or LiCl (138 mM) in bath solution?

E. How do the potencies of some known SCN5a blockers compare in the ICR flux assay versus patch clamp?

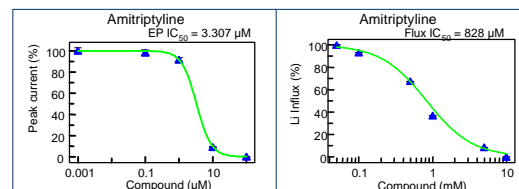


Figure 5. The IC₅₀ of amitriptyline in the presence of NaCl (138 mM) in patch clamp was observed to be 3.3 μM in comparison to 828 μM in flux assay using LiCl (140 mM).

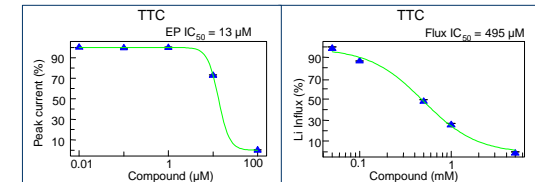


Figure 6. The IC₅₀ of TTC in the presence of NaCl (138 mM) in patch clamp was observed to be 13μM in comparison to 495 μM in flux assay using LiCl (140mM)

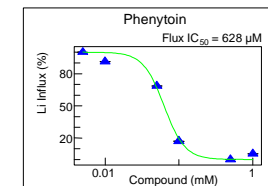


Figure 7. The IC₅₀ of Phenytoin in the presence of LiCl (140 mM) in flux assay was observed to be 628 μM. Data from patch clamp was not available.

F. Does the rank order differ in flux assay from patch clamp?

Answer: The assay is being optimized and the rank order would be drawn after screening a panel of drugs.

V. Conclusion

Here, the preliminary data on Li⁺ Influx Assay has been shown to identify compounds that change ion flow of Li⁺ through sodium channels. The blockers of Na⁺ channels can be easily identified using Aurora Biomed's ICR 8000 and Flux Assay. As described in the literature, flux assays can be optimized to identify channel activity for both ligand-gated and voltage-gated ion channels. Although more experiments are required, it is evident that ICR technology is adaptable to many ion channel HTS needs.

VI. Acknowledgements

We are thankful to **Dr Hali Hartmann** for the HEK/SCN5a cell line. We are also thankful to **Mr. Ali Shabar** and **Paul Qu** for technical help.

VII. References

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