ASIC CHANNEL SCREENING

HIGH THROUGHPUT SCREENING ASSAY FOR ACID-SENSING ION CHANNELS

Introduction

Therapeutic Importance: Acid-sensing ion channels (ASICs) are members of the amiloride-sensitive epithelial Na^+-channel/degenerin superfamily (ENaC/DEG). These ligand-gated ion channels are activated by protons, which are released during tissue acidosis (increase in extracellular acidity). Tissue acidosis, which is evidenced by a drop in extracellular pH, occurs in many acute and chronic pain conditions including inflammation, angina, stroke, ischemic heart disease, arthritis, cancer and traumatic injuries. Protons have been shown to induce pain in humans and it is believed this signal may be mediated by ASICs. This family of channels consists of six isoforms: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4. Of the six ASIC subunits ASIC1 (1a/1b) and ASIC3 are attractive targets for the treatment of pain since primary sensory neurons are highly enriched with these channels. These primary afferent fibers are nociceptors that detect and signal painful sensations from the periphery to the brain. ASIC1a and ASIC3 are activated by an acidity range (pH 7.0-6.0) observed in several acute and chronic pain conditions. Moreover, ASIC3 is particularly sensitive to lactic acidosis and thus is considered to be an important component of acid-induced pain response during cardiac and muscle ischemia.

Screening Assay: Like many sodium ion channels, ASICs represent challenging targets for high throughput screening. To overcome this issue, Aurora Biomed's proprietary flux assay approach was successfully applied to this novel ion channel target. 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. In these flux assays, tracer ions not normally present in biological systems are utilized to minimize background noise.

Methods

Li^+ as a Tracer: ASIC channels being mainly sodium selective, Li^+ was used as the tracer ion of choice to monitor ASIC activity.

The assay is based on Li^+ influx into ASIC-expressing CHO cell lines, in response to acidic extracellular pH conditions (Figure 1). The initial validating experiments included pH dose-response curves as well as testing of known ASIC channel modulators described in the literature. All the experiments were performed in duplicate/triplicate with 8 concentrations.

Assay Protocol: The basic protocol consisted in pre-incubating the cells for 10 minutes in 198µL of Wash Buffer, alone or spiked with 2 µL of a 100X solution of the test compound before the activation step. The latter was achieved by replacing the Wash Buffer with 198 µL of the Li^+ containing Channel Open Buffer (adjusted to the desired acidic pH) in the presence or absence of test compound, as described above. After a 10-minute incubation at room temperature, the cells were washed and lysed

Analysis: The intracellular content of Li^+ in each well (100 µL samples) was measured by Aurora Biomed's ICR series.

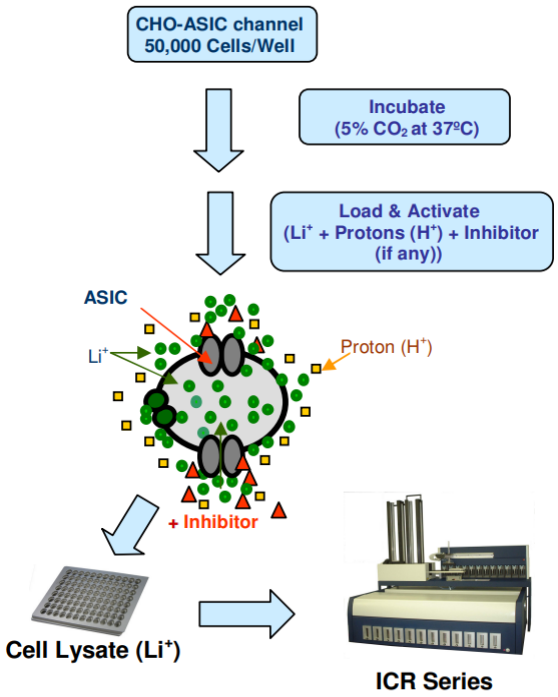
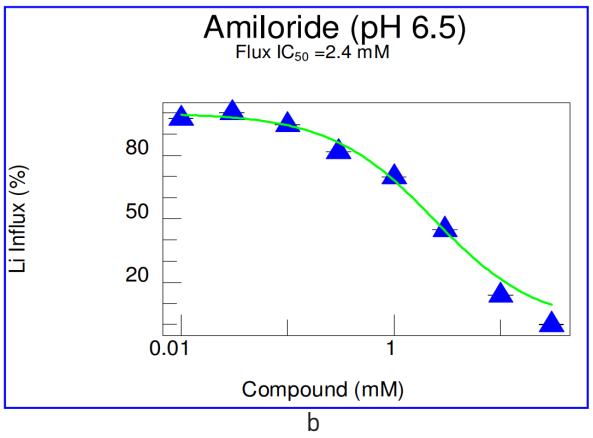


Figure 1. Aurora Biomed's Li^+ -based ASIC screening assay

Results

Experimental conditions were established to obtain a robust signal to noise ratio and provide an adequate window of detection of ASIC-modulating substances. The assay did not display significant variability as indicated by a Z' factor above 0.5 and no major cell loss was observed during the liquid handling. Results obtained with ASIC active substances were similar to those reported in the literature. Thus, the divalent ion Zn^{2+} was also seen to increase the sensitivity of some ASIC-channels that resulted in a shift of the pH activation toward less acidic and more physiological values (Figure 2). Furthermore, the assay was also capable of detecting ASIC inhibitors such as amiloride and gadolinium. The IC_{50} determinations for both substances at different pH are presented in Figure 3.



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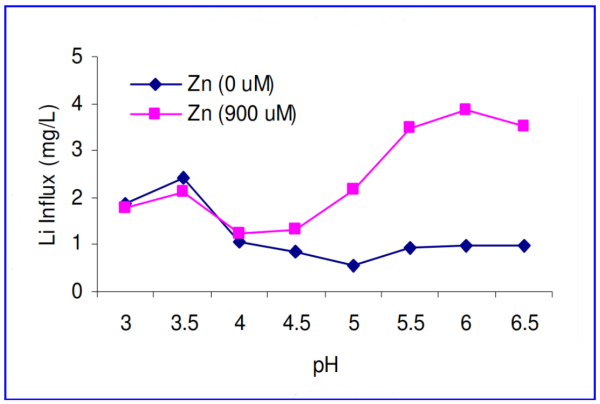

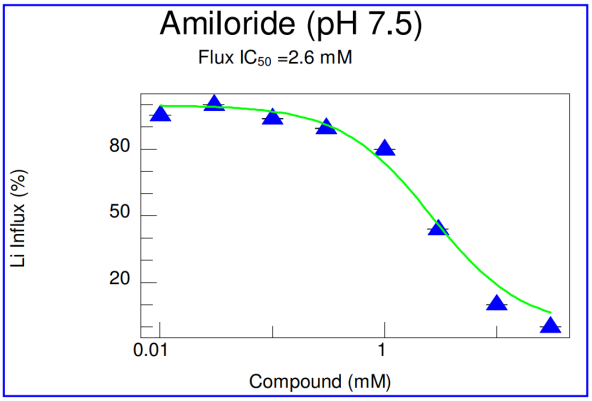

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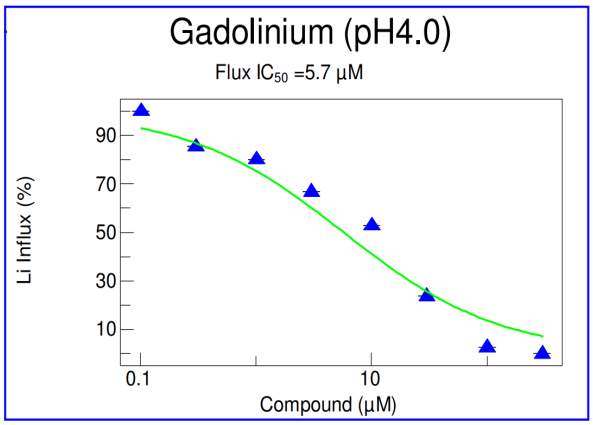
Figure 2. Activation of ASIC channels in response to increasing proton concentrations (pH 6.5 to 3.0), and modulation of pH-sensitivity by Zn^{2+}



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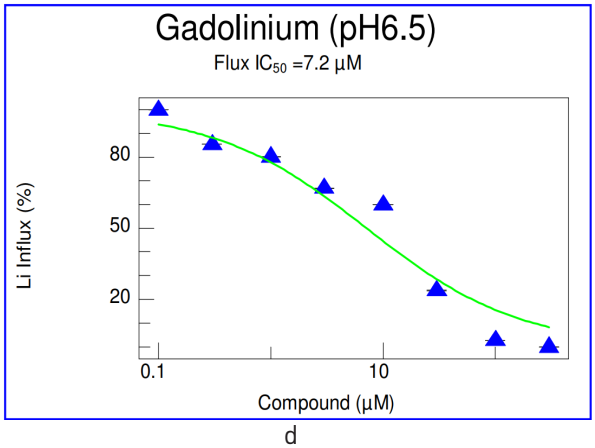

d

Figure 3(a-d). IC_{50} values of gadolinium and amiloride at different pH.

References

1. Lingueglia E. Acid-sensing ion channels in sensory perception. JBC 2007 ;282(24):17325-17329
2. Baron A, Schaefer L, Lingueglia E, Champigny G, Lazdunsky M: Zn^{2+} and H^{+} are coactivators of acid sensing ion channels. JBC 2001;276(38):35361-35367.
3. Babinski K, Catarsi S, Biagini G, Séguéla P. Mammalian ASIC2a and ASIC3 subunits co-assemble into heteromeric proton-gated channels sensitive to Gd3+ . JBC 2000 ;275(37):28519-28525.
4. Zhang JH, Chung TD, Oldenburg KR: A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen 1999;4(2):67-73.
5. Wang K, McIlvain B, Tseng E, Kowal D, Jow F, Shen R, Zhang H, Shan Q J, He L, Chen D, Lu Q, Dunlop J: Validation of an atomic absorption rubidium ion efflux assay for KCNQ/M-channels using the Ion Channel Reader 8000. Assay Drug Dev Technol 2004;2(5):525-534.