Introduction

Neuro2A cells are a mouse neuroblastoma cell line used extensively to investigate neuronal differentiation, axonal growth and cell signalling pathways. They are also used as an expression system for studying ion channels. Neuro2A cells have been shown to endogenously express NaV channels, predominantly NaV1.1, but also NaV1.2, 1.3 and 1.4 (Lou et al., 2003), mechanosensitive channels Pieza 1 (Coste et al., 2010; Rotordam et al., 2019), purinergic receptors (Vetter & Lewis, 2010) and glutamate receptors (Van der Valk & Vijverberg, 1990). In this study, we used the medium and high throughput automated patch clamp devices, Patchliner and SyncroPatch 384, to record different ion channels endogenously expressed in Neuro2A cells.

NaV recorded from Neuro2A is TTX sensitive

Figure 1: A voltage-gated sodium current was recorded from Neuro2A cells. A We calculated a Vm of activation of -19.4 ± 0.6 mV (n = 8 cells) using a Boltmann equation. B The NaV current was completely blocked by nm concentrations of TTX with an IC50 of 4.8 ± 0.5 nM (n = 9 cells). Multiple concentrations of TTX were added to each well of the NPC-16 chip of the Patchliner and complete concentration response curves were constructed from 9 individual cells. C & D The NaV current was also blocked by Tetracaine (C) and Lidocaine (D) with an IC50 of 11.2 ± 2.9 µM (n = 12) and 64 ± 15 µM (n = 12), respectively, holding potential was -100 mV.

NaV pharmacology: Tetracaine and Lidocaine

Figure 2: TRP channels are expressed in Neuro2A cells. External solution was heated in the pipette of the Patchliner and rapidly applied to the cells. A heat-activated response was seen in 8/8 cells at 42°C. Following this, different TRP channel activators were applied. No activation was seen with capsicain in 8/8 cells, whereas all cells (8/8) responded to GSK(101769A) and 4/8 cells responded to 2-APB. Shown are raw current traces from an example cell (left) and the timecourse of the experiment (right). This indicates that TRPV4 and in some cells, TRPV3, is expressed in Neuro2A cells but not TRPV1.

Conclusions

- Neuro2A cells can be used on automated patch clamp devices with success rates of 60-80% for 1 GO seals
- Voltage-gated Na currents were recorded with a Vm of activation of -19.4 ± 0.6 mV and blocked by nM concentrations of TTX
- The NaV current was also blocked by lidocaine and tetracaine at µM concentrations
- A heat activated response was measured after application of external solution at 42°C. This was mediated by TRPV4 or TRPV3 but not TRPV1
- Piezo1 was recorded from Neuro2A cells on the SyncroPatch 384 following application of Yoda1
- Neuro2A cells are a suitable cell type for investigating endogenous NaV currents, as well as Piezo1 and some TRP channels.