

Cardiac Na_v1.5 recorded on the Patchliner®: reliable biophysical characterization and pharmacology

Nanon Technologies GmbH, Munich

Summary

Voltage gated sodium channels (Na_v) are important elements of action potential initiation and propagation in excitable cells. The channels are activated upon a depolarization of the membrane. Their activation leads to further depolarization of the membrane which constitutes the upstroke of the action potential.

The Na_v1.5 channel, encoded by the SCN5A gene, is a voltage-gated sodium (Na_v) channel found in skeletal muscle and heart¹. It is TTX insensitive with an IC₅₀ in the micromolar range¹. Na_v1.5 is responsible for the upstroke of the cardiac action potential in both ventricular and atrial myocytes² and is therefore critical for generation and propagation of the cardiac action potential in human heart. Block of this channel can lead to prolongation of the QRS interval of the electrocardiogram (ECG) and can have profound effects on the rate of cardiac depolarization and conduction velocity, thus causing potentially dangerous cardiac arrhythmias^{3,4}. Furthermore, effects of Na_v1.5 inactivation can modify cardiac repolarization⁴. Given the importance of this channel in maintaining cardiac function, it has become an important target in compound safety screening.

Na_v currents generally activate very fast (within 1-2 ms) upon depolarization of the membrane. Hence, a good and stable access resistance is critical for high quality pharmacological patch clamp recordings. Also, for patch clamp experiments, accurate delivery of compound to the cell is a prerequisite for accurately reproducible concentration response curves (CRCs

and therefore, an extremely important aspect in the assay.

Using the new Patchliner® with integrated amplifiers from Elements, we could accurately record current-voltage relationships and pharmacological modulation by the local anaesthetic tetracaine.

Results

Na_v1.5 stably expressed in CHO cells (Charles River, #CT6007) were used on the Patchliner® with success rates exceeding 85% for recordings. Current responses to increasing voltage steps were used to construct current-voltage plots. The data was analyzed using DataControl PL analysis software for the Patchliner®. The curves were fit with a Boltzmann equation and the IV plot for an example cell is shown in Figure 1. The average V_{half} of individual plots was -31.4 ± 3.1 mV (n = 10) in good agreement with the literature⁵.

In addition, pharmacology of Na_v1.5 was investigated using the local anaesthetic tetracaine. Using a single step voltage

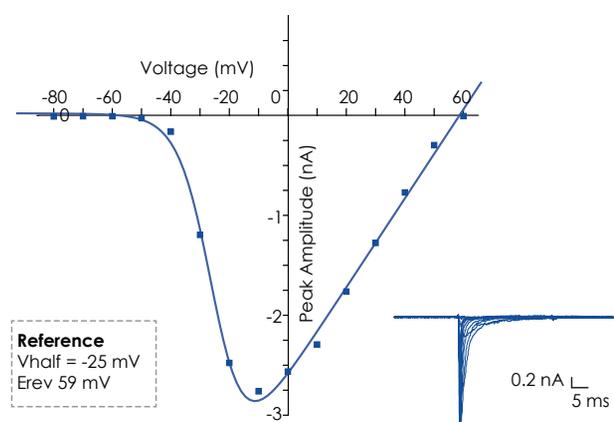


Figure 1: Current-voltage plot for Na_v1.5 expressed in CHO cells. The IV curves for each individual well were fit with a Boltzmann equation. Representative IV curve and traces from an example cell are shown.

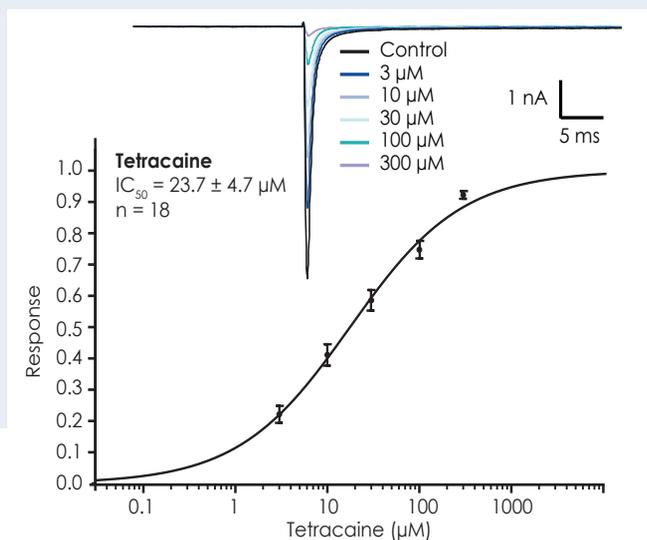


Figure 2: Tetracaine inhibited $\text{Na}_v1.5$ in a concentration-dependent manner. The CRC for an average of 18 cells is shown and traces from an example cell are shown above. The IC_{50} for tetracaine was $23.7 \pm 4.7 \mu\text{M}$ ($n = 18$ cells). Data was acquired using PatchlinerControl and analyzed using DataControl PL for the Patchliner[®].

protocol repeated every 10 s, cumulative additions of tetracaine could be made on each cell and individual CRC constructed. The CRC for tetracaine is shown in Figure 2 for an average of 18 cells (3 chips pooled). The corresponding raw traces of an example cell are also shown.

In order to investigate state-dependence of compounds, a more depolarized holding potential can be used and the CRC repeated with different holding potentials. Alternatively different voltage protocols can be used, e.g. a double-step protocol where the V_{half} of activation is used as the voltage between the two steps and the CRC can be generated for both peaks. A shift in the CRC on the second peak indicates state-dependence of the compound. Where compounds are highly state-dependent, even small differences in holding potential can have a large influence on IC_{50} , therefore accurate voltage control is essential.

In summary, the Patchliner[®] was used to record $\text{Na}_v1.5$ expressed in CHO cells and biophysical properties, such as V_{half} of activation, and pharmacology of a local anaesthetic could be recorded and analyzed using PatchlinerControl and DataControl PL for the Patchliner[®]. The Patchliner[®] with integrated amplifiers is an excellent tool for studying $\text{Na}_v1.5$ for biophysical characterization and pharmacology.

References

1. Gellens, M.E., *et al.*, 1992. PNAS. 89: 554-558
2. Nerbonne, J.M. & Kass, R.S. 2005. Physiol. Rev. 85: 1205-1253
3. Bowes, J., *et al.*, 2012. Nat. Rev. Drug. Disc. 11: 909-922
4. Erdemli, G., *et al.*, 2012. Front. Pharmacol. doi: 10.3389/fphar.2012.00006
5. Bean, B.P., *et al.*, 1983. J.Gen. Physiol. 81: 613 - 642 Jepps, T.A., *et al.* 2021. Front. Physiol. 12:679317

Methods

Cells

CHO cells stably expressing $\text{Na}_v1.5$ (Catalog #CT6007) were supplied by Charles River.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard protocols. Cells were resuspended in Nanion's external recording solution (#08 3001) and stored in the CellHotel of the Patchliner[®] before being dispensed into each well of the NPC-16 chip. Nanion's standard internal (#08 3008) and external solutions (#08 3001 and #08 3004) were used for recordings (solution compositions available upon request). For IV plots, voltage was increased in 10 mV steps from -80 mV to 60 mV from a holding potential of -100 mV. For pharmacology of tetracaine, a single step to 0 mV from a holding potential of -100 mV was used. Data was analyzed using DataControl PL. IV plots were fit with a Boltzmann equation and CRC plots with a Hill equation. Data is given as mean \pm S.E.M.

