Application Note

Channel: $hNa_V1.5$
Cells: CHO; iCell® Cardiomyocytes[2]
Tools: Patchliner

$I_{Na\text{-}Late}$ recorded from CHO cells and hiPSC-CMs on Nanion´s Patchliner

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iCell® Cardiomyocytes[2] kindly provided by Fujifilm Cellular Dynamics International.
CHO cells kindly provided by Charles River.

Summary

The voltage-gated Na^+ channel 1.5 ($Na_V1.5$) is encoded by the SCN5A gene and is responsible for the rising phase of the cardiac action potential (AP). The $Na_V1.5$ channel is comprised of a pore-forming α subunit and auxillary β subunits[1]. When the cardiac cell membrane depolarizes, $Na_V1.5$ opens for a short time allowing an influx of Na^+ ions resulting in the upstroke of the action potential. During the action potential, these channels can recover from inactivation and re-open resulting in a sustained current termed $I_{Na\text{-}Late}$. Although this current is substantially smaller than the peak Na^+ current ($I_{Na\text{-}Peak}$), it is active during the plateau phase and therefore contributes to AP morphology[2]. There is a growing body of evidence that increased $I_{Na\text{-}Late}$ can have a pathophysiological role in acquired heart diseases such as myocardial ischemia and heart failure[3]. $I_{Na\text{-}Late}$ is elevated in several pathological conditions which could result in Na^+-overload in these cells. A number of loss or gain-of-function mutations in the SCN5A gene have been identified which lead to changes in the magnitude or duration of $I_{Na\text{-}Peak}$ or $I_{Na\text{-}Late}$ resulting in fatal arrhythmias[1]. $I_{Na\text{-}Late}$ is a potential drug target to treat cardiac disorders such as angina, heart failure and arrhythmia[4]. It is also an important target in safety pharmacology as enhancement of $I_{Na\text{-}Late}$ is pro-arrhythmic[5].

In this study the Patchliner was used to record $I_{Na\text{-}Late}$ from CHO cells and hiPSC-CMs. $I_{Na\text{-}Late}$ was recorded using the voltage protocol specified by CiPA and activated using ATX-II. $I_{Na\text{-}Late}$ could be recorded from CHO cells stably expressing $Na_V1.5$ and blocked by lidocaine. $I_{Na\text{-}Late}$ could also be detected in iCell® Cardiomyocytes[2] and blocked by ranolazine.

Results

CHO cells expressing $Na_V1.5$ were used on the Patchliner. The voltage protocol shown at the top of Figure 1 was used to elicit $I_{Na\text{-}Late}$ as recommended by the CiPA study. The peak inward current during the ramp part of the protocol was used for analysis. ATX-II was used to activate $I_{Na\text{-}Late}$. Figure 1 shows the raw current traces of $I_{Na\text{-}Peak}$ and $I_{Na\text{-}Late}$ in the presence of ATX-II and block by lidocaine. Figure 2 shows the concentration response curve to lidocaine for the peak (**A**) and late (**B**) current. The IC_{50} was approximately 7 times lower on the late current compared with the peak current.

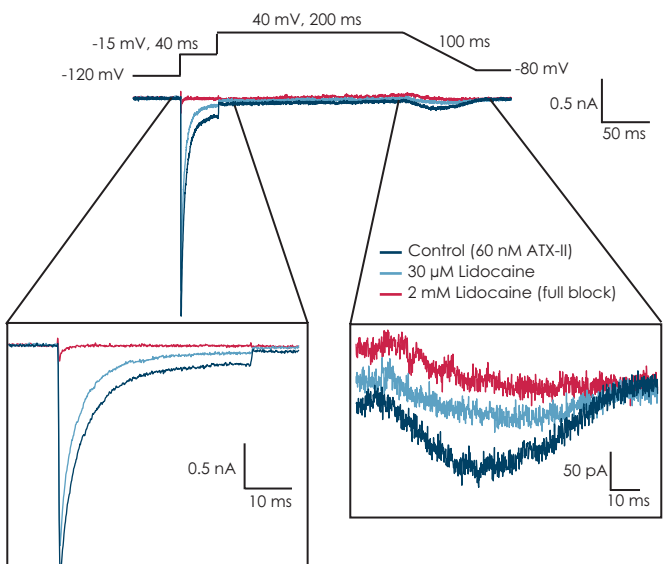

Figure 1:
$I_{Na\text{-}Late}$ was activated by 60 nM ATX-II using the voltage protocol shown at the top. The current was blocked by lidocaine. A single concentration of lidocaine was applied per well followed by full block by 2 mM lidocaine (red). Recordings performed at 35°C. The insert shows the peak (left) and late (right) current enlarged.

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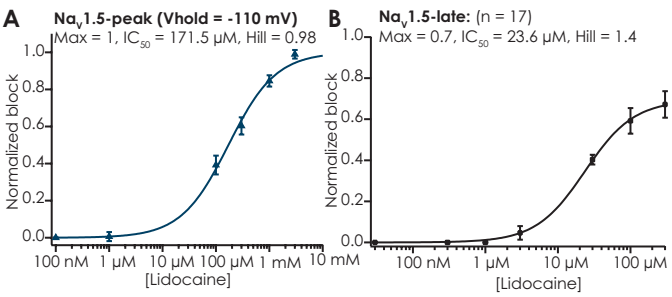

Figure 2:
A Concentration response curve for lidocaine on $Na_V1.5$ peak current and **B** late current. Single point concentration response curves were performed and the concentration response curves calculated over several plates. The IC_{50} for lidocaine was approximately 7 times lower on the late current compared with the peak current.

We also investigated whether we could record $I_{Na\text{-}Late}$ in hiPSC-CMs. Using the same voltage protocol as shown in Figure 1, and ATX-II at a concentration of 100 nM, we could detect a small but statistically significant $I_{Na\text{-}Late}$ in iCell® Cardiomyocytes[2]. The $I_{Na\text{-}Late}$ current was blocked by the selective blocker, ranolazine. Figure 3 shows $I_{Na\text{-}Peak}$ and $I_{Na\text{-}Late}$ current recorded from an example iCell® Cardiomyocyte[2] showing activation by ATX-II and block by ranolazine. The current elicited by ATX-II was statistically significant for n = 5 cardiomyocytes.

In conclusion, the Patchliner, in combination with an $hNa_V1.5$ expressing CHO cell line (Charles River) or hiPSC-CMs, is a robust electrophysiological tool for investigating effects of compounds on $I_{Na\text{-}Late}$ for drug discovery and safety testing.

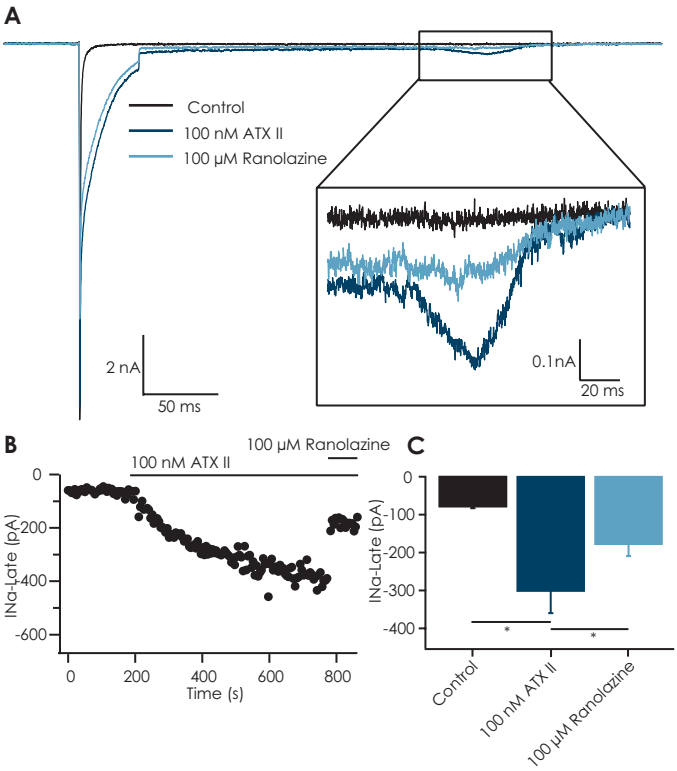

Figure 3:
A Example traces from an iCell® Cardiomyocyte[2] showing $I_{Na\text{-}Peak}$ and $I_{Na\text{-}Late}$. **B** Timecourse of the experiment. **C** Average current amplitude for 5 cells. $I_{Na\text{-}Late}$ was statistically significantly enhanced by ATX-II and blocked by ranolazine ($P<0.05$, student's paired t test).

References

1. Han D., *et al*., 2018. Exp. Biol. Med. 243: 852-863.
2. Horvath B., & Bers D.M. 2014. ESC Heart Failure. 1: 26–40.
3. Maier L.S., & Sossalla S. 2013. JMCC. 61: 44-50.
4. Makielski J.C. 2016. Trends Cardiovasc Med. 26(2): 115–122.
5. Belardinelli L., et al. 2013. JPET. 344: 23–32.

Methods

Cells

CHO cells stably expressing $hNa_V1.5$ (Catalog # CT6007) were supplied by Charles River.
iCell® Cardiomyocytes[2] (Catalog # CMC-100-012-000.5) were supplied by Fujifilm Cellular Dynamics International.

Cell culture

CHO cells and hiPSC-CMs were cultured and harvested according to Nanion's standard cell culture protocols for cell lines and stem cells, respectively.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner with standard solutions. Currents were elicited using a voltage step/ramp protocol (see Figure 1) repeated every 10 s in the presence of 60 nM (CHO) or 100 nM (hiPSC-CMs) ATX-II applied via the external solution. Experiments were performed at 35°C (CHO) or room temperature (hiPSC-CMs). The peak current recorded during the ramp phase of the voltage protocol was used for analysis of $I_{Na\text{-}Late}$.

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