Biophysical and pharmacological properties of K_V7 channels: an automated patch clamp study

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1 Introduction

$K_V7.1$ channels are primarily expressed in cardiac cells whereas $K_V7.2$, $K_V7.3$, and $K_V7.5$ are widely distributed in neuronal and primary sensory cells[1]. $K_V7.4$ is expressed in the cochlear and is essential for normal hearing. $K_V7.1$ mediates the cardiac I_{Ks} current, a slowly activating delayed rectifier potassium current. The channel consists of the pore-forming a subunit (KCNQ1) and a modulatory β subunit (KCNE1) and is an important ion channel for ventricular repolarization[1,2]. Decreases in I_{Ks} due to genetic mutations cause Long QT syndrome, a potentially fatal cardiac disorder[2,3]. Equally, compounds which inhibit I_{Ks} can also induce potentially fatal arrhythmia. In neurons, K_V7 channels mediate the M-current, a slowly activating, non-inactivating, time- and voltage-dependent potassium current, which regulates resting membrane potential (RMP), controls action potential firing rates and neuronal excitability. In adult neurons, K_V7 channels are most frequently composed of $K_V7.2$ and $K_V7.3$ subunits as heterotetramers[4]. Mutations in the genes encoding $K_V7.2/7.3$ subunits cause severe functional disruption of the channel and lead to developmental and epileptic encephalopathy[3]. These channels are potential therapeutic targets for human hyperexcitability diseases. For example, M-channel openers such as retigabine are effective anticonvulsants and antiepileptics in a range of *in vitro* and *in vivo* models. In addition, $K_V7.2/7.3$ modulators may provide clinical benefit in other neuropsychiatric diseases, including pain, migraine, anxiety, and neurodegenerative diseases[1,3,4].

2 Automated patch clamp recordings of $K_V7.1$/KCNE1

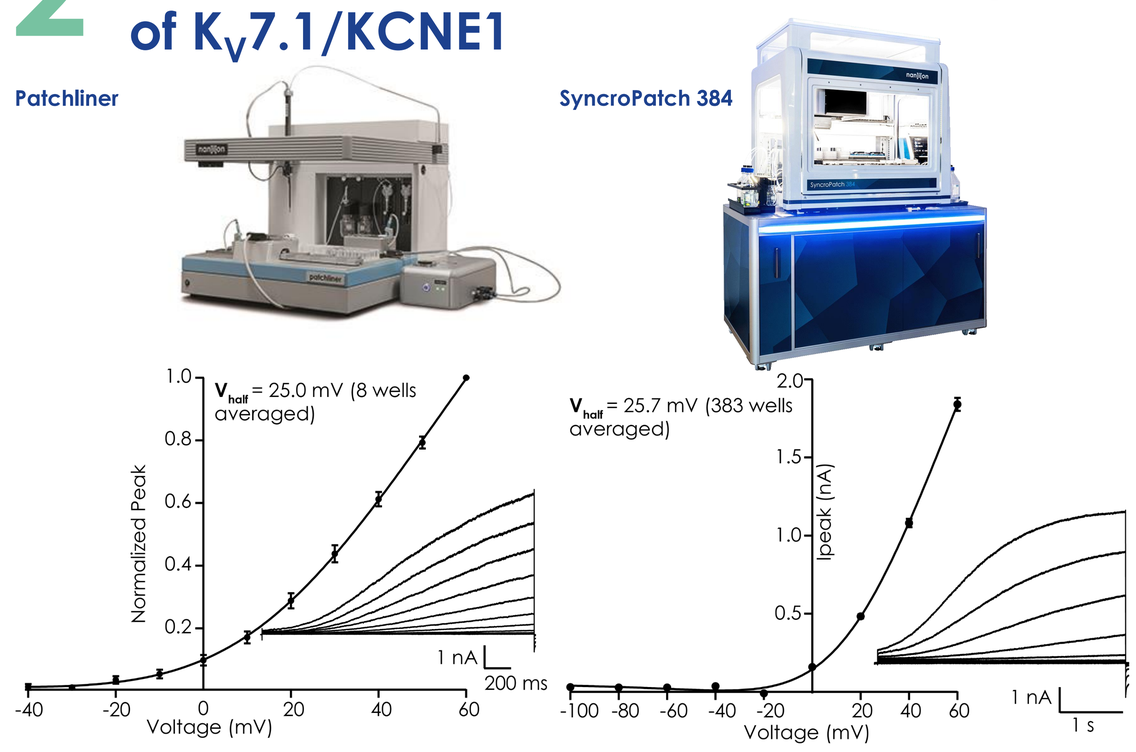


Figure 1: (Left) $K_V7.1$/KCNE1 expressed in CHO cells recorded on the Patchliner and (right) on the SyncroPatch 384. The I_{Ks} current was elicited using increasing voltage steps. In both instruments the voltage activation threshold was approximately -20 mV in good agreement with the literature[5]. The V_{half} of activation was comparable between the two instruments.

3 Biophysical properties and stability of $K_V7.2/7.3$-mediated currents

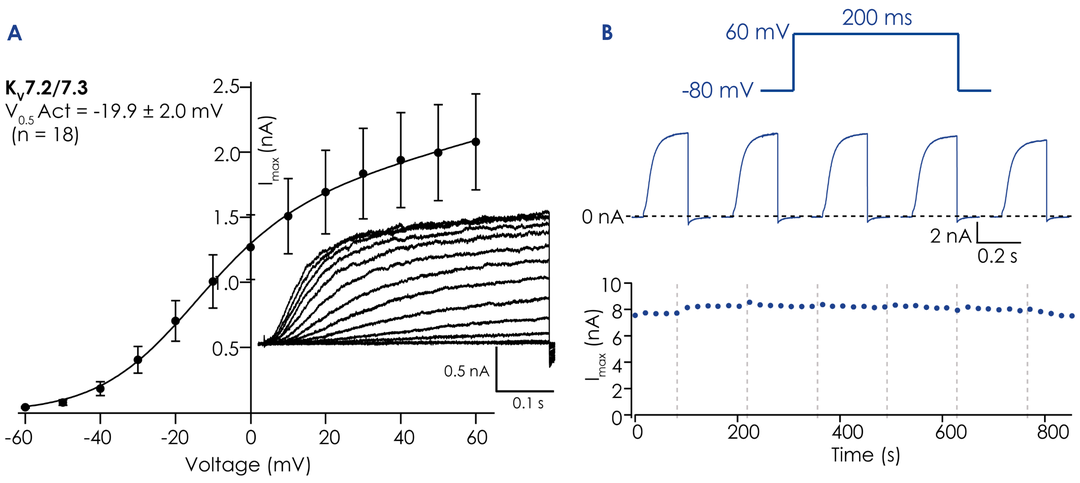
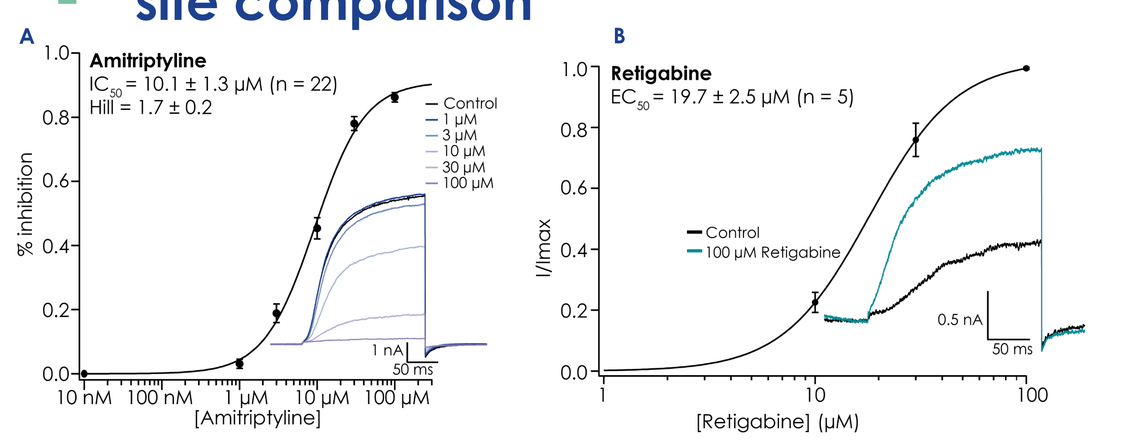


Figure 2: Current-voltage relationship and stability of $K_V7.2/7.3$ recordings. A Current-voltage plot of an average of 18 HEK cells expressing $K_V7.2/K_V7.3$ (experiments performed at ApconiX). Traces from an exemplar cell are shown in the inset. Individual IV curves were fit with a Boltzmann equation and the average V_{half} of activation was calculated to be -19.9 ± 2.0 mV (n = 18) in excellent agreement with the literature[6]. At a second site (Nanion HQ, Munich), the V_{half} of activation was calculated to be -22.2 ± 1.8 mV (n = 16), in excellent agreement with the data generated at ApconiX and with the literature[6]. **B** Using a single step protocol to 60 mV from a holding potential of -80 mV (top) repeated every 20 s, current amplitude was stable over time. Representative $K_V7.2/K_V7.3$ traces are shown in the middle and the corresponding timecourse at the bottom. Recordings were stable for at least 15 minutes.

4 Pharmacology of $K_V7.2/7.3$ and cross-site comparison



Parameter	ApconiX, UK	Nanion HQ
V_{half}	-19.9 ± 2.0 (n = 18)	-22.2 ± 1.8 (n = 16)
IC_{50} amitriptyline (µM)	6.9 ± 1.2 (n = 8)	10.2 ± 1.3 (n = 22)

Table 1: V_{half} and amitriptyline IC_{50} values across 2 sites

Figure 3: Pharmacology of $K_V7.2/7.3$. A $K_V7.2/7.3$ was blocked by amitriptyline in a concentration-dependent manner. The concentration response curve for an average of 22 cells is shown and the IC_{50} calculated using a Hill equation for 22 individual plots was 10.1 ± 1.3 µM (n = 22) in excellent agreement with the literature[7]. The same experiment was run at a different site revealing a similar IC_{50} of 6.9 ± 1.2 µM (n = 8, Table). The values were not statistically different (unpaired Student's t test, p>0.05). **B** Retigabine is an anticonvulsant that enhances $K_V7.2/7.3$-mediated responses by shifting the voltage-dependence of activation to more hyperpolarized potentials[8,9,10]. In our experiments, retigabine enhanced $K_V7.2/7.3$ responses in a concentration-dependent manner. An EC_{50} of 19.7 ± 2.5 µM (n = 5) was calculated when the data was normalized to the maximum concentration (100 µM) and fit with a Hill equation.

5 Enhancement of $K_V7.4$ by XEN1101

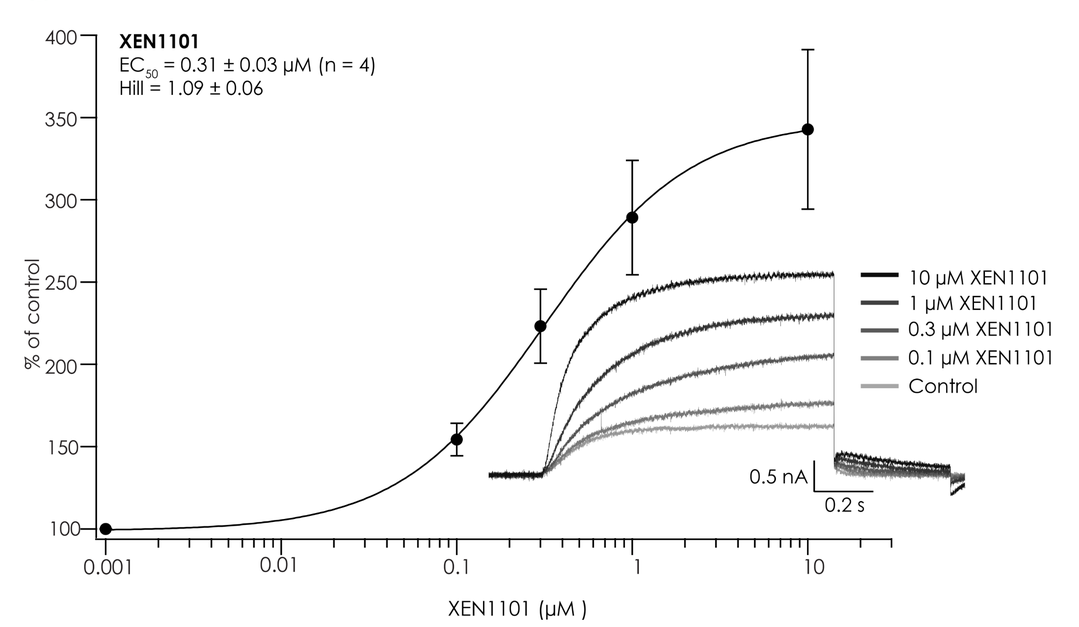


Figure 4: $K_V7.4$ expressed in CHO cells was recorded on the Patchliner. XEN1101 is a Kv7 channel opener which has entered clinical trials for the treatment of epilepsy, major depressive disorder (MDD), with potential to treat other neurological disorders[11]. In our experiments, XEN1101 enhanced $K_V7.4$-mediated responses in a concentration-dependent manner. The data was normalized to control (no XEN1101) and fit with a Hill equation revealing an EC_{50} = 0.31 ± 0.03 µM (n = 4). The highest concentration (10 µM) enhanced the current response by 343 ± 49%(n = 4) compared to control.

6 Summary

- $K_V7.1$ (KCNQ1/KCNE1)-mediated currents were recorded on the Patchliner and SyncroPatch 384 showing a similar V_{half} of activation
- $K_V7.2/7.3$ (KCNQ2/KCNQ2) was recorded at two different sites. V_{half} of activation was -20 mV, amitriptyline blocked with a similar IC_{50} at the two different sites and retigabine enhanced $K_V7.2/7.3$ currents
- $K_V7.4$ (KCNQ4)-mediated responses were recorded on the Patchliner and enhanced by XEN1101

References

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