INVESTIGATING THE EFFECTS OF LAMOTRIGINE ON HCN CHANNELS: A TEST CASE FOR A NEW MINIATURIZED PATCH-CLAMP AMPLIFIER

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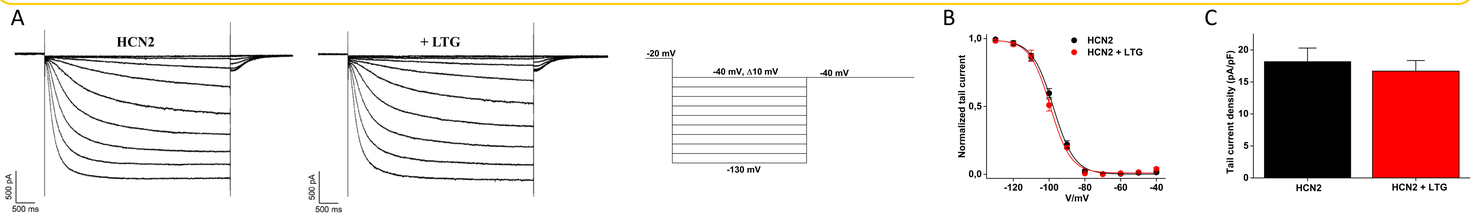
Introduction

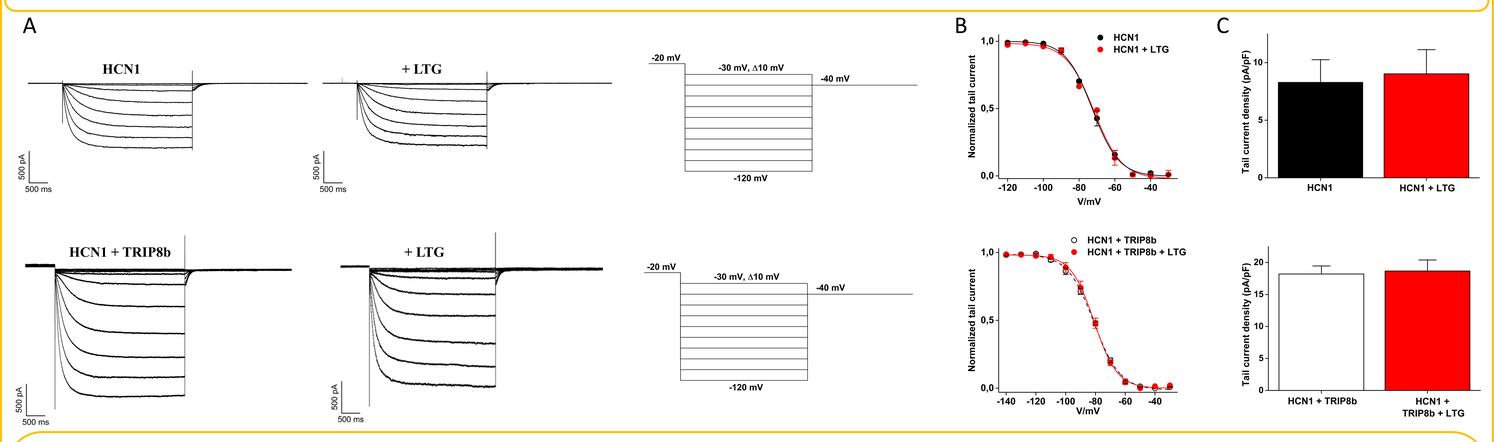
There are contrasting reports in the literature on the effects of lamotrigine (LTG), a widely used anticonvulsant drug, on modulation of native I_h currents in neurons. Poolos et al. [1] reported an increase in current mainly due to a positive shift in the activation curve of the channel, while Peng et al. [2] reported an increase in maximal current but no shift in the voltage dependency. To better understand these results we expressed the main components of the I_h current in neurons, HCN1 and HCN2, separately in HEK293T cells and measured their functional properties in the absence or presence of LTG. In addition, we assessed LTG effects obtained in the presence of the neuronal HCN channel auxiliary subunit TRIP8b [3]. Measurements were performed using a new all-in-one integrated amplifier prototype, designed and developed by Elements SRL, the ePatch. The miniaturized ePatch amplifier is USB plug-n-play and integrates the analog front-end with multiple gains stage, the analog-to-digital converter and filters all into a single small headstage without any external bulky case.

LAMOTRIGINE HAS NO EFFECT ON VOLTAGE DEPENDENCE AND CURRENT DENSITY OF HCN2 CHANNEL



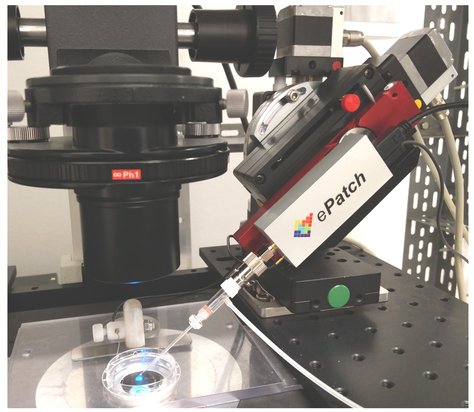
A, Representative current traces of HCN2 in control solution (left) and in the presence of 100µM LTG (20 min application in the bath solution, centre). The applied voltage protocol is shown (right). Currents were recorded in whole-cell configuration, at R.T., 24h after transfection of HEK293T cells with mHCN2 cDNA. **B**, Activation curves obtained from tail currents collected at –40mV. Fitting the Boltzmann equation to data yielded the following values of half activation voltage ($V_{1/2}$) and inverse slope factor (s): HCN2 (black line), $V_{1/2}$ = -97.6 ± 0.4 mV, s =5.9 ± 0.3 mV mV, N=5; HCN2 + 100µM LTG (red line), $V_{1/2}$ = -99.4 ± 0.6 mV, s = 5.7 ± 0.6 mV mV, N=5. **C**, Mean normalized tail current density of HCN2 (black) 18.2 ± 2.1 pA/pF, N=5 and HCN2 + 100µM LTG (red) 16 ± 1.65 pA/pF, N=5. Data are presented as mean ± SEM. All data were acquired at 5 kHz using ePatch amplifier.

LAMOTRIGINE HAS NO EFFECT ON VOLTAGE DEPENDENCE AND CURRENT DENSITY OF HCN1 CHANNEL ALONE OR IN COMPLEX WITH ITS REGULATORY SUBUNIT TRIP8b



A, Representative current traces of HCN1 alone (top) and cotrasfected with the regulatory subunit TRIP8b (bottom) in control solution and in the presence of 100µM LTG applied for 20 min in the bath solution. The applied voltage protocol is shown (right). Currents were recorded in whole cell configuration, at R.T., 24h after transfection of HEK293T cells with hHCN1 and mTRIP8b 1a4 (where indicated) cDNAs. **B,** Activation curves obtained from tail currents collected at –40mV. Fitting the Boltzmann equation to data yielded the following values of half activation voltage ($V_{1/2}$) and inverse slope factor (s). Top, HCN1 (black line), $V_{1/2}$ = -71.7 ± 1.2 mV, s= 7.8 ± 1.5 mV, N=4; HCN1 + 100µM LTG (red line), $V_{1/2}$ = -72.6 ± 0.6 mV, s= 7.4 ± 0.5 mV, N=4. Bottom, HCN1 + TRIP8b (black dash line), $V_{1/2}$ = -81.1 ± 0.5 mV, s= 8.8 ± 0.5, N=5; HCN1 + TRIP8b + 100µM LTG (red line), $V_{1/2}$ = -80.9 ± 0.4 mV, s= 7.7 ± 0.3 mV, N=5. **C,** Mean normalized tail current density. Top, HCN1 (black), 8.3 ± 1.95 pA/pF, N=4 and HCN1 + 100µM LTG (red), 9 ± 2.1 pA/pF, N=4 . Bottom, HCN1 + TRIP8b (black), 18.2 ± 1.3 pA/pF, N=5; HCN1 + 100µM LTG (red), 18.7 ± 1.7 pA/pF, N=5. Data are presented as mean ± SEM. All data were acquired at 5 kHz using ePatch amplifier.

ePatch details

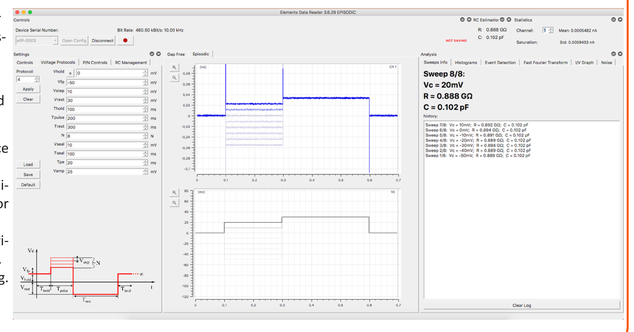


The miniaturized **ePatch** amplifier integrates in a small headstage the low-noise amplifier, the pulse generator and the digitizer into only 42 x 18 x 78 mm and it is connected directly to the USB port of a laptop without the need of any other external bulky digitizer. It has the following features:

- Open input (RMS) noise: 150 fA rms @ 1kHz - 480 fA rms @ 10 kHz - 4 pA rms @ 100 kHz
- Current ranges: ±200pA (Gain 2.25GΩ), ±2nA (Gain 225MΩ), ±20nA (Gain 22.5MΩ), ±200nA (Gain 2.25MΩ)
- Max sampling rate: 200 kS/s
- Parametric voltage protocols for pulse generator in the range of ± 500 mV
- C-fast - C-slow - R-series - P/N compensations
- C fast compensation range: 0 - 11 pF
- R series compensation range: 0 - 10 MΩ
- Auto electrodes voltage offset fine compensation
- Continuous C-membrane and R-seal estimation
- Programmable digital I/O
- Zap pulse
- Digital filters: cut-off frequencies in the range between 62,5 Hz and 100 KHz
- USB powered
- Dovetail or rod bar mounting
- Compatible with standard pipette holders (BNC or SMA connectors)

The ePatch is controlled by the **EDR3 EPISODIC software** interface that enables an easy control of the amplifier and displays data in real time, both in the gap-free or episodic modalities. Input current ranges, bandwidth, voltage stimulus and electrode offset compensation can all be easily selected and modified. Moreover it has the following peculiarities:

- real-time data analysis (I/V graph for channel conductance and reversal potential, FFT, etc.)
- continuous membrane resistance and capacitance monitoring (R and C values can be automatically saved for every sweep)
- digital LabBook to manually record events and experimental details such as used cell line, buffer solutions, etc.
- data outputs saved in different formats: .dat (e.g. Matlab™), .abf (e.g. pClamp™)
- available for Windows and Mac OS



Conclusions

The results of our experiments show that lamotrigine does not have a direct effect on HCN channels, suggesting that the reported increases in I_h activity in neurons may result from indirect effects of the drug on other cellular components. This finding may also explain the contrasting results observed following lamotrigine application to native I_h currents in distinct neuronal types, and implies that lamotrigine may not necessarily act to increase I_h conductance in all cells and conditions. The recordings demonstrate the high-quality data acquired by the ePatch, which are comparable to those acquired by state-of-the-art amplifiers in terms of signal to noise ratio and data content.

Acknowledgments

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References

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