The ePatch amplifier is controlled by the EDR3 EPISODIC software tool (R and C values can be automatically saved for every sweep)

• Open input (RMS) noise: 115 fA rms @ 1kHz
• Max sampling rate: 200 kS/s
• Open input (RMS): ±200nA (Gain 2.25MΩ), ±2nA (Gain 225MΩ)
• C fast compensation range: 0–250 pF,
• C series compensation range: 0–100 pF
• Programmable digital I/O
• Dovetail or rod bar mounting
• USB powered
• Coolant or water not requiring
• Software real-time data analysis (S/2) graph for channel conductance and neuron potential, FFT, etc.
• Real-time membrane resistance, access resistance and capacitance monitoring software tool (R and C values can be automatically saved for every sweep)
• Data outputs saved in different formats: .dat (.Matlab®), .edg (.pcl method)

Our results show that D and E helices of HCN4 channels are involved in the control of IHCN4 affinity suggesting that this regulatory mechanism is conserved among the three most reported isoforms (HCN1, HCN2, HCN4). Further patch clamp experiments will be performed on HCN4 mutant in order to clarify the individual role of D and E helices. Notably, a deletion of D helix was studied in 2002 by another research group showed an activation shift of -60 mV in 0 mM cAMP. To extend this work to the cardiac channel isoform, we test the role of helices D and E in HCN4 using the miniaturized ePatch amplifier, developed and designed by Elements SRL, the ePatch. The miniaturized ePatch amplifier is USB plug-in-ready and integrates the analog front-end with multiple stages, the analog-to-digital converter and filter all into a single small headstage without any external bulky digitizers.

HCN4 WT + 1µM cAMP shift V1/2 = 103.2 ± 1.2 mV, s= 11.3 ± 0.9, N=5; HCN4 WT + 30µM cAMP (red dashed line), V1/2 = 91.7 ± 1.1 mV, s= 10 ± 1.1 mV, N=5. HCN4 WT + 1µM cAMP (black dashed line), V1/2 = 91.6 ± 1.1 mV, s= 10 ± 1.1 mV, N=5. 

Deletion of the C-terminus downstream of DE helices does not affect CAMP response of HCN4 channel expressed in HEK293T cells

Deletion of the C-terminus upstream of the DE helices decreases cAMP response of HCN4 channel expressed in HEK293T cells

A. Representative current traces of HCN4 WT (top) and ΔC-term mutant (bottom) in control solution and in the presence of sub-saturating concentration (1µM) of cAMP in the pipette solution. The potential applied at -120 mV is highlighted in red. The applied voltage step protocol is shown (top, right). Currents were corrected in whole cell configuration, at RT, 24h after transfection of HEK293T cells with HCN4 WT and ΔC-term mutant cRNA. B. Activation curves, obtained from tail currents collected at -120 mV at 20°C labelled as ΔDE helices (D and E helices). For each curve we calculated the half-activation voltage (V1/2) and inverse slope factor (nH). C. Mean shift induced by 1µM or 30µM cAMP on HCN4 WT and ΔC-term mutant calculated from activation curves shown on the left. Shift values are as follow: HCN4 WT + 1µM cAMP V1/2 = 122 ± 1.5 mV, HCN4 WT + 30µM cAMP V1/2 = 122 ± 1.5 mV; HCN4 ΔDE + 1µM cAMP V1/2 = 103 ± 2 mV, HCN4 ΔDE + 30µM cAMP V1/2 = 103 ± 2 mV. **p<0.01, ***p<0.001 using unpaired test. All data were acquired at 5 kHz (SR) using ePatch amplifier, saved in .abf format and analyzed offline.

References

L. Lee and R. Mackinnon 2017, Cell 168:1111