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Introducing simulated I_{K1} and leak compensation into human iPSC-cardiomyocytes using dynamic clamp on an automated patch clamp system

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Backround: Dynamic clamp is a powerful tool involving injection of real-time simulated membrane currents into patch clamped cells. This technique has been employed in conventional patch clamping to inject inward rectifier I_{K1} current into human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).

Methods: hiPSC-CMs are attractive cell types, because of their unlimited availability and human origin. However, I_{K1} is usually expressed at lower levels in those cells, resulting in a more depolarized membrane potential than in adult cardiomyocytes (CM). This might affect also the function of all other voltage-gated ion channels. Introducing simulated I_{K1} into hiPSC-CMs may improve K^+-conductance in these cells and ensure that they represent a viable alternative to the scarcely available dissociated adult human ventricular CMs. Another limitation of hiPSC-CMs is that the ratio of seal resistance (R_{seal}) to membrane resistance (R_m) is small due to smaller cell capacitance and typically slightly lower R_{seal} using automated patch clamp systems. Thus, the dissipation of the membrane potential is more pronounced compared to patch clamp recordings of adult CMs. To correct for that, we implemented R_{seal} compensation (RSC) mechanism to inject current, compensating for the potential dissipation through R_{seal}. While calculation of the required current depending on the instantaneous membrane potential is not complex, estimating and tracking R_{seal} during a whole-cell patch clamp experiment is.

Results: our results show that virtual I_{K1} can be successfully injected into hiPSC-CMs in up to 4 cells simultaneously and that R_{seal} can be correctly compensated avoiding overcompensation. Our approach results in more stable resting membrane potentials and improved action potential (AP) shape with respect to duration stability. Increased I_{K1} resulted in AP shortening and acceleration of the upstroke. We measured native Ba^{2+}-sensitive I_{K1} in voltage clamp mode in approximately 50% of these cells, but I_{K1} was small, on average 1.98±0.42 pA/pF (mean ± SEM). Adding a Ca^{2+} channel activator (BayK 8644), or blocker (Nifedipine) caused an increase and decrease of the AP duration, respectively.

Conclusion: in conclusion, combining dynamic clamp and R_{seal} compensation with automated patch clamping resulted in an enhanced, medium-throughput platform for safety pharmacology.