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MEETING ABSTRACT

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Investigating the pharmacology of L-type Ca^{2+} channels using stable cell lines and brain activity patterns

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Background: L-type Ca^{2+} channels (LTCCs) respond to membrane depolarization by opening their conductive pore and thereby allow Ca^{2+} ions to enter the cell. $Ca_V1.3$, one of the two main brain LTCC isoforms, has been recently implicated in the pathophysiology of Parkinson's disease. LTCCs are particular sensitive towards organic Ca^{2+} channel blockers (CCBs), such as the dihydropyridine isradipine, which mostly act in a state-dependent way. Thereby, parameters such as the shape and frequency of the depolarizing stimuli can strongly influence drug responsiveness. Since usually cardiac myocyte-like long and infrequent square pulses are used to study the pharmacology of CCBs, IC_{50} values for CCB-mediated inhibition during neuronal activity patterns are unknown. Therefore, we generated stable cell lines of $Ca_V1.3$ channel constructs capable of reproducibly quantifying the pharmacological activity of CCBs under physiological recording conditions (2 mM Ca^{2+}) using neuronal activity stimuli.

Methods: Inducible cell lines stably expressing human $Ca_V1.3$ long and short splice variant ($hCa_V1.3_L$, $hCa_V1.3_S$) were generated using the Flp-In™ T-REx™ system (together with β3 and α2–δ1) and the expression of full-length α1 subunits biochemically confirmed using western blot analysis. Biophysical and pharmacological channel properties were measured by the patch-clamp whole-cell technique and compared with the same constructs transiently transfected in tsA-201 cells using the Ca^{2+} phosphate precipitation method.

Results: Stable cell lines expressing $hCa_V1.3_L$ and $hCa_V1.3_S$ exhibit regularly large current amplitudes (500–2000 pA) and similar biophysical properties compared to transiently transfected constructs. However, a ~8 mV left-shift of the Ca^{2+} (I_{Ca}) current–voltage relationship towards more hyperpolarized voltages as well as faster I_{Ca} inactivation were observed for both constructs in the stable cell lines. We show that the high expression level allow reliable recordings of $Ca_V1.3$ currents even under stimulation conditions (such as action-potential-like command voltages) that induce substantial steady-state inactivation of I_{Ca}. At present, we are also generating cell lines stably expressing $rbCa_V1.2_S$ and $Ca_V1.3$ channels containing *CACNA1D* mutations causing human diseases.

Discussion: In conclusion, our data demonstrate that inducible cell lines stably expressing LTCCs are a feasible approach for extensive drug screening under physiological recording conditions (2 mM Ca^{2+}) where large and consistent currents are required.

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