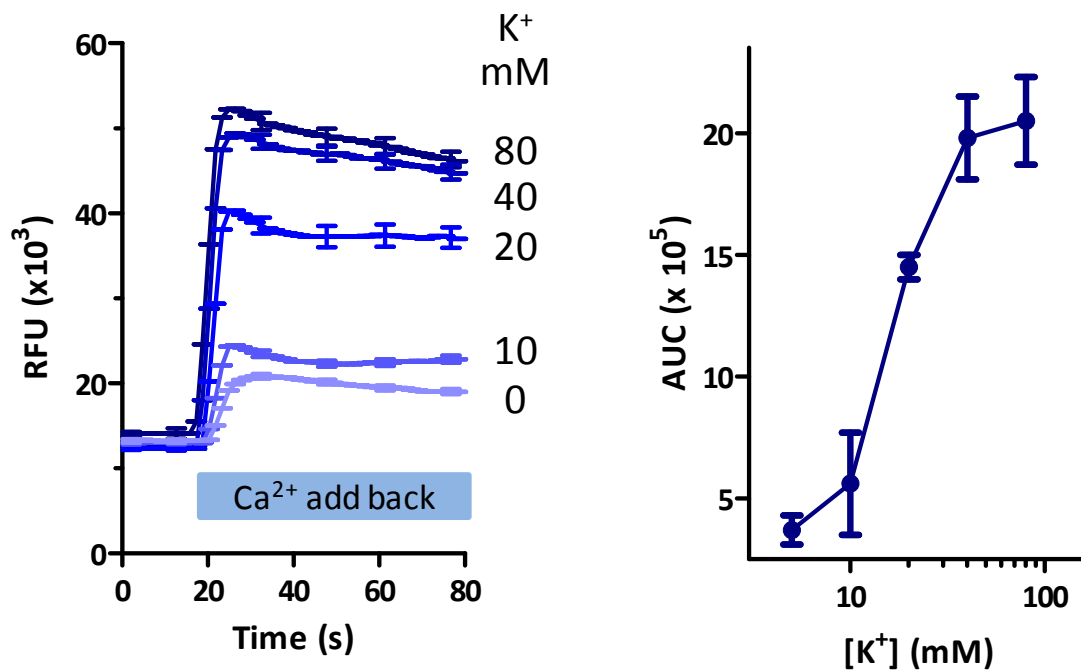


hCa_v1.2 L-type voltage-gated Ca²⁺ channel Fluorescence Assay

- **Assay** hCa_v1.2 fluorescence assay
- **Channel** L-type voltage-gated Ca²⁺ channel, α 1C co-expressed with K_{ir}2.3
- **Gene Name** *CACNA1C*
- **Synonyms** Cardiac or smooth muscle calcium channel; dihydropyridine receptor
- **Assay format** 96-well FLEXstation optical assay – intracellular Ca²⁺
- **Cell Host** Human Embryonic Kidney Cells (HEK-293)
- **Stimulus** Ca²⁺ addback; compounds incubated under depolarized conditions
- **Controls** Nimodipine, 0.3% DMSO

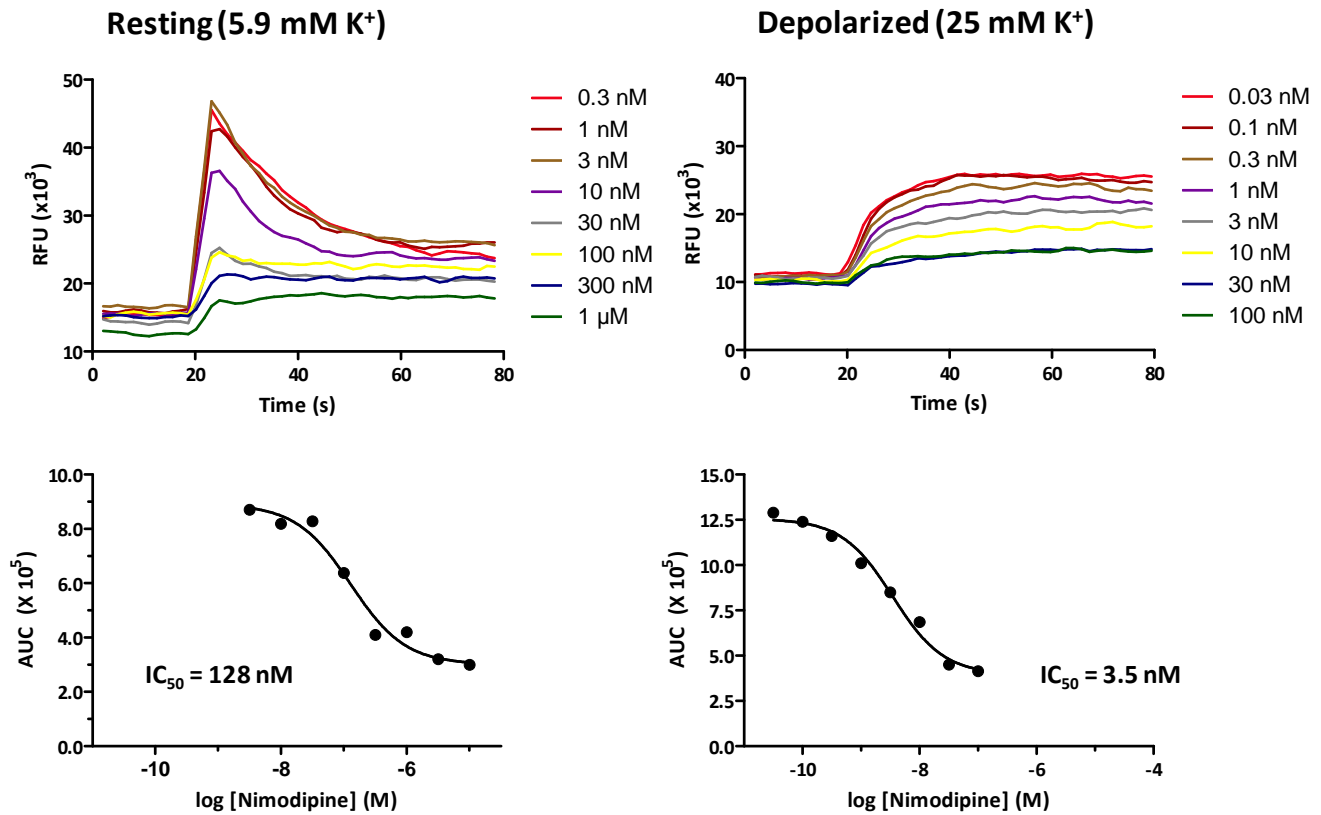


Assay

Fluo4 fluorescence measurements of K⁺-induced changes in intracellular Ca²⁺ (FLEXstation) in L-type Ca²⁺ channel expressing HEK-293 cells.

Background Voltage-gated L-type Ca^{2+} channels are widely distributed in excitable tissues and mediate excitation-contraction coupling, electrical and 2nd messenger signal transduction as well as neurotransmitter/hormonal release. A number of widely used L-type Ca^{2+} channel blockers, that include dihydropyridines (DHP), phenylalkylamines (PA) and benzothiazepines (BZT), are clinically useful antihypertensives. Off-target block of L-type Ca^{2+} channels include bradycardia, disruption of cardiac electrical conduction, blood pressure effects and muscle weakness.

Pharmacology State-dependent Inhibition of L-type Ca^{2+} channels: Nimodipine



Reference Compound data

	Class	Depolarized IC ₅₀ (nM)
Nimodipine	DHP	9.6 ± 1.7
Nitredipine	DHP	4.6 ± 0.4
Nifedipine	DHP	13.1 ± 1.6
Verapamil	PA	1540 ± 229
Diltiazem	BTZ	3117 ± 739

Mean ± SEM (n=10-12)

Why Essen? Many electrophysiological and optical assays of L-type Ca²⁺ channels lack sensitivity to small molecule L-type Ca²⁺ channel blockers. Ten- to 100-fold higher IC₅₀ values compared to therapeutic free plasma concentrations and effects of L-type Ca²⁺ channel blockers in smooth muscle assays have been observed. Assay conditions used in our optical Ca_v1.2 assay produce a state-dependent evaluation of compound effects on this important target, with sensitivity for reference compounds in the low nM to low μM range. The throughput of this optical assay can support rapid evaluation of late-stage candidates or SAR studies to reduce the potential liability of interactions with L-type Ca²⁺ channels.

References Xia, M. *et al.*, State-dependent inhibition of L-type calcium channels: cell-based assay in high-throughput format, *Analytical Biochemistry* (2004), 327 (1):74–81.

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