B'SYS GmbH

$Ca_V3.1$ / $Ca_V3.3$ patch - clamp assay

Specification Sheet

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1. INTRODUCTION

1.1 T-Type Calcium channels

T-type Ca^{2+} channels were originally called low-voltage-activated (LVA) channels because they can be activated by small depolarizations of the plasma membrane. In many neurons Ca^{2+} influx through LVA channels triggers low-threshold spikes, which in turn triggers a burst of action potentials mediated by Na+ channels. Burst firing is thought to play an important role in the synchronized activity of the thalamus observed in absence epilepsy, but may also underlie a wider range of thalamocortical dysrhythmias. In addition to a pacemaker role, Ca^{2+} entry via T-type channels can directly regulate intracellular Ca^{2+} concentrations, which is an important second messenger for a variety of cellular processes. Molecular cloning revealed the existence of three T-type channel genes. The deduced amino acid sequence shows a similar four-repeat structure to that found in high-voltage-activated (HVA) Ca^{2+} channels, and Na^{+} channels, indicating that they are evolutionarily related. Hence, the alpha1-subunits of T-type channels are now designated Ca_V3. Although mRNAs for all three Ca_V3 subtypes are expressed in brain, they vary in terms of their peripheral expression, with $Ca_V3.2$ showing the widest expression. The electrophysiological activities of recombinant Cav3 channels are very similar to native T-type currents and can be differentiated from HVA channels by their activation at lower voltages, faster inactivation, slower deactivation, and smaller conductance of Ba^{2+}.

2. MATERIAL AND METHOD

CHO cells were transfected with $Ca_V3.1$ or $Ca_V3.3$ cDNA. During the electrophysiological experiment they were continuously perfused with buffer solution: 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, 10 mM HEPES, pH (NaOH) 7.40. The Pipette was filled with a solution containing: 135 mM CsF, 0.1 mM $CaCl_2$, 5 mM NaCl, 54 mM CH_3O_3SCs, 2.25 mM $MgCl_2$, 2 mM Na_2ATP, 2.5 mM Creatine, 2.5 mM Phosphocreatine (disodium), 2.5 mM Pyruvate (free acid), 2.5 mM Oxalacetate, 17 mM HEPES, 7.5 mM EGTA, pH (CsOH) 7.2. After the seal was obtained cells were clamped to -80 mV.

3. RESULTS

3.1 Biophysical Characterization

$Ca_V3.1$ and $Ca_V3.3$ channel were biophysically characterized. The IV curves and the inactivation curves were recorded using voltage protocols given below. IV curves were normalized to the largest current amplitude, inactivation curves were normalized to the maximum of a Boltzmann function fit to the individual cells. Data were averaged and for the inactivation curves $V_{0.5}$ and the K values were determined.

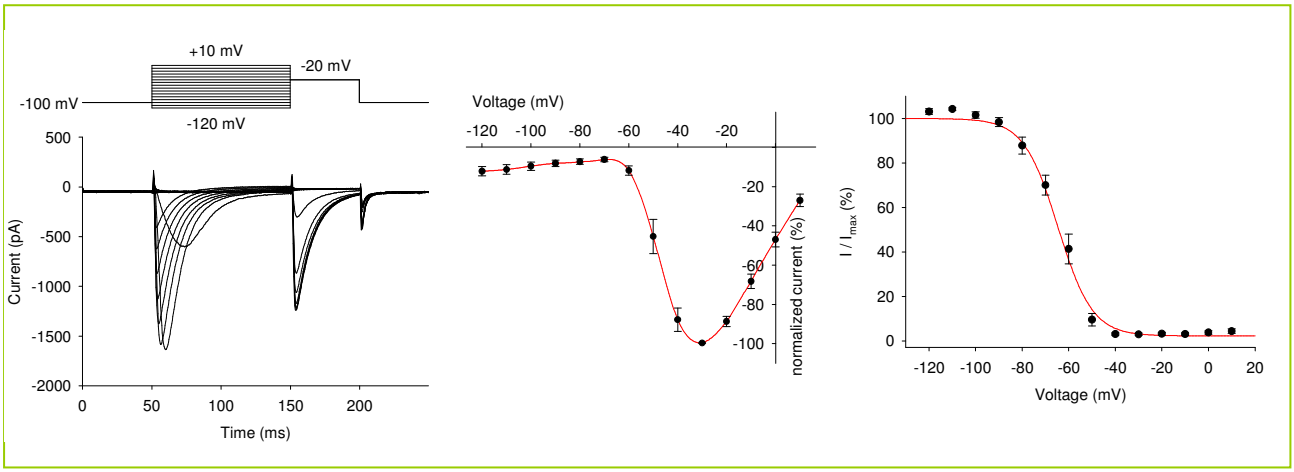

Fig.1: Biophysical characterization of $Ca_V3.1$: left: representative current recording and applied voltage protocol, middle: IV curve, right: inactivation curve.

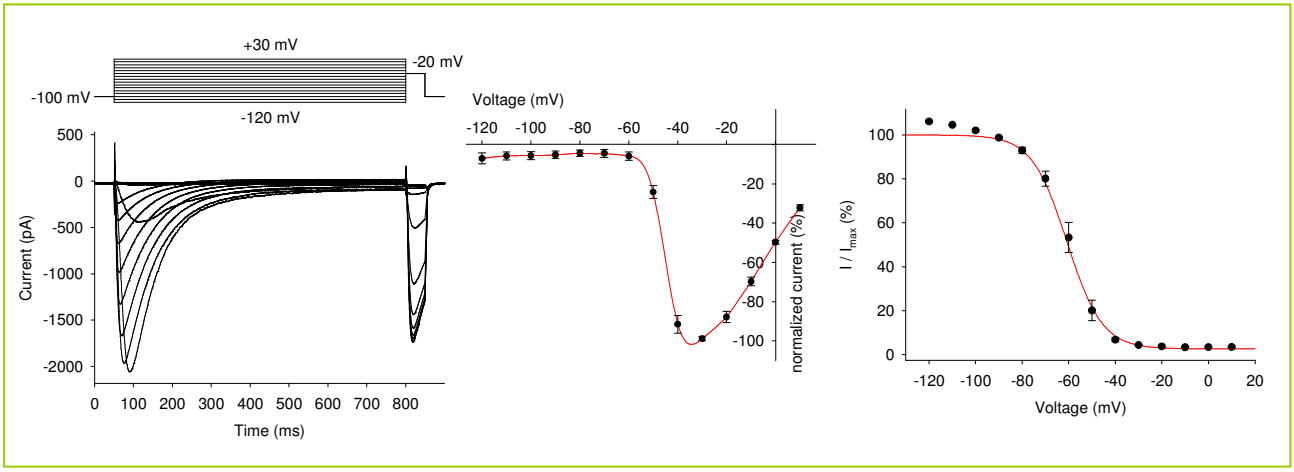

Fig.2: Biophysical characterization of $Ca_V3.3$: left: representative current recording and applied voltage protocol, middle: IV curve, right: inactivation curve.

Table 1: Summary of $V_{0.5}$ and K values

	$V_{0.5}$ value	k value
$Ca_V3.1$	-64.59 ± 1.80 mV	7.09 ± 0.18
$Ca_V3.3$	-60.67 ± 1.96 mV	7.13 ± 0.02

3.2 Pharmacological Characterization

Mibefradil was used to validate the pharmacological properties of $Ca_V3.1$ and $Ca_V3.3$ channels. The following concentrations were tested:

$Ca_V3.1$: 5 nM, 50 nM, 500 nM and 5000 nM

$Ca_V3.3$: 50 nM, 500 nM, 1500 nM, 5000 nM and 50000 nM

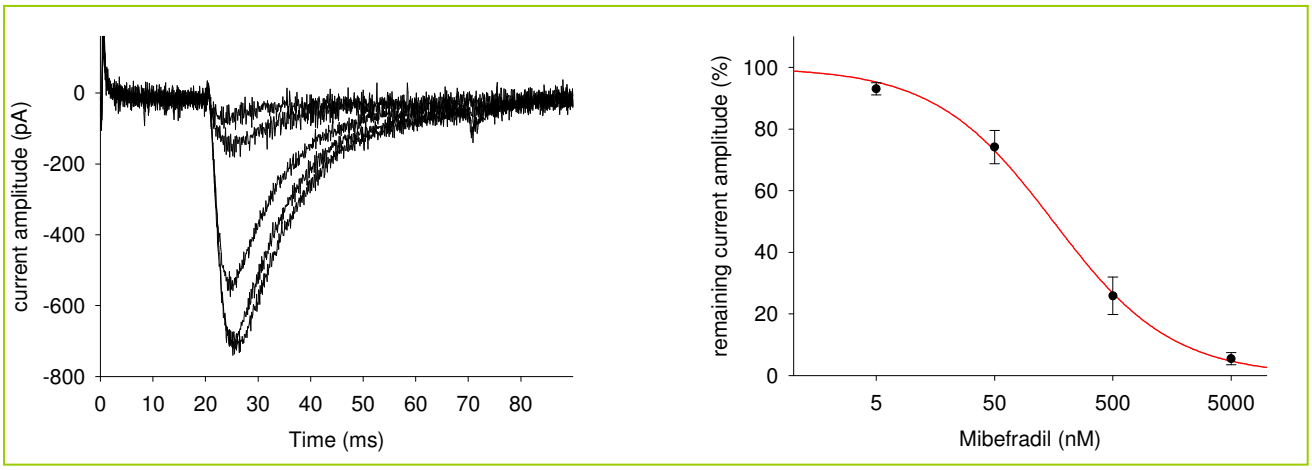

Fig.3: Effect of Mibefradil on $Ca_V3.1$ channels: left: representative current recording, right: dose response curve

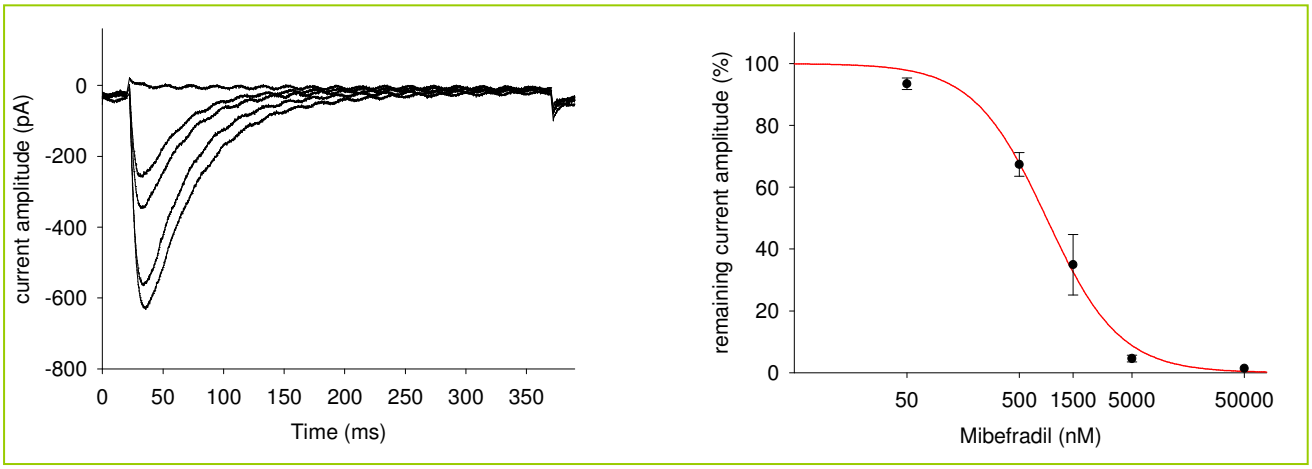

Fig.4: Effect of Mibefradil on $Ca_V3.3$ channels: left: representative current recording, right: dose response curve

Table 2: Summary of IC_{50} values for Mibefradil

	IC_{50} value	Hill coefficient	IC_{50} Literature
$Ca_V3.1$	156.95 nM	0.87	251 nM – 1000 nM
$Ca_V3.3$	872.02 nM	1.33	1585 nM

4. ASSAY FLEXIBILITY

B'SYS offers its clients a high degree of flexibility. Client designed stimulation or application protocols can be transferred and validated within a short time.

5. CONTACT INFORMATION

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