

Supporting Online Materials

MOLECULAR DETERMINANT FOR SPECIFIC Ca/Ba SELECTIVITY PROFILES OF LOW AND HIGH THRESHOLD Ca²⁺ CHANNELS

Cens, T., Rousset, M., Kajava, A. & Charvet, P.

These supplementary informations contain

- 3 supplementary tables and their legends.
- 4 legends for supplementary figures 1-4
- 4 supplementary figures

Supplementary Table I

Effects of mutations at the DCS locus on Cd²⁺ block of Ba²⁺ current and current reversal potentials in 10 mM Ba²⁺ or Ca²⁺.

Mutants channel	EC50 (Cd²⁺) in μM	E_{Rev} (Ba²⁺) in mV	E_{Rev} (Ca²⁺) in mV
Ca _v 2.3 WT [12]	2±1	65±2	73±2
Ca _v 2.3(DSED) [10]	3±1	65±1	74±2
Ca _v 2.3(TSQN) [11]	10±1	65±1	75±1
Ca _v 2.3(DEEE) [7]	2±1	66±1	74±2

EC50 for the inhibition of Ba²⁺ currents by Cd²⁺ were obtained from dose-response curves realized in 10 mM Ba²⁺ bathing solution using increasing concentration of Cd²⁺. As in figure 4C.

Reversal potential were measured on current-voltage curves realized using voltage-ramps from -80mV to +80 mV (2.5 V/s) in 10 mM Ba²⁺ or Ca²⁺ bathing solutions.

Supplementary Table II

The mutations at the DCS locus marginally affect the activation parameters

Mutants channel	Ba²⁺		Ca²⁺	
	V _{act} (mV)	k (mV)	V _{act} (mV)	k (mV)
Ca _v 2.3 WT [12]	-3±1	-4.4±0.3	5±2	-4.5±0.4
Ca _v 2.3(DSED) [16]	-6±3	-4.3±0.6	-1±1	-3±0.2
Ca _v 2.3(TSQN) [15]	4±1*	-6.1±0.3	10±2*	-7.2±0.6
Ca _v 2.3(DEEE) [15]	-8±2*	-3.6±0.5	3±2	-3.9±0.3
Ca _v 2.3(DEQN) [6]	-2±1	-5.6±0.2	9±2	-5.5±0.1
Ca _v 2.3(GGGG) [5]	-3±3	-7.1±0.5	6±3	-7.4±0.6
Ca _v 2.3(AAAA) [6]	-1±2	-6.5±0.1	7±2	-7.1±0.2
Ca _v 2.1 WT [13]	-11±1	-3±0.5	-3±1	-2.9±0.3
Ca _v 2.1(TEQE) [11]	-9±1	-4.1±0.1	-2±1	-3.7±0.2

Activation parameters were obtained from the fit of the current voltage-curve realized in either 10 mM Ba²⁺ or 10 mM Ca²⁺ bathing solutions using the equation:

$$I/I_{max} = G \cdot (V - E_{Rev}) / (1 + \exp((V - V_{act})/k))$$

where I is the current amplitude measured during voltage steps varying from -80 to +50 mV, I_{max} is the peak current amplitude measured at the minimum of the current-voltage curve, G is the normalized macroscopic conductance, E_{Rev} is the apparent reversal potential, V_{act} is the potential for half-activation, V is the value of the voltage step and k a slope factor. A student t-test has been used to compare each mutant values with the control Ca_v2.3, and differences were considered statistically significant (marked by *) when P<0.05.

Supplementary Table III

The mutations at the DCS locus marginally affect the inactivation parameters

Mutants channel	Ba²⁺			Ca²⁺		
	V _{in} (mV)	k _{in} (mV)	R _{in} (%)	V _{in} (mV)	k _{in} (mV)	R _{in} (%)
Ca _v 2.3 WT [14]	-22±1	8.5±0.3	53±4	-11±1	8.9±0.5	56±3
Ca _v 2.3(DSED) [7]	-22±2	8.5±0.8	60±3	-14±1	7.2±0.5*	55±6
Ca _v 2.3(TSQN) [22]	-21±1	8.8±0.4	58±3	-20±1*	9.6±0.5	62±6
Ca _v 2.3(DEEE) [11]	-20±1	8.7±0.9	56±3	-14±1	8.2±1.0	59±3
Ca _v 2.3(DEQN) [11]	-16±3*	9.4±0.8	59±4	-12±5	9.4±1.5	62±7
Ca _v 2.3(GGGG) [5]	-25±1	10.2±0.6*	47±4	-15±5	10.7±1.1	47±3
Ca _v 2.3(AAAA) [5]	-28±2*	9.3±0.6	38±3*	-20±1*	10.4±1.1	49±4
Ca _v 2.1 WT [13]	-11±1	5.7±0.4	58±2	-4±1	3.9±0.5	64±4
Ca _v 2.1(TEQE) [8]	-7±2	7.9±0.6	34±3*	-7±3	6.2±1.9	45±7

Isochronal steady-state inactivation curves (2.5 seconds of conditioning voltage followed by a 400 ms test pulse to +10 mV) were fitted using the following equations:

$$I/I_{max}=R_{in}+(1-R_{in})/(1+\exp((V-V_{in})/k))$$

where I is the current amplitude measured during the test pulse at +10 mV for conditioning voltages varying from -80 to +50 mV, I_{max} is the current amplitude measured during the test-pulse for a conditioning voltage to -80 mV, V_{in} is the potential for half-inactivation, V is the voltage, k_{in} is the slope factor and R_{in} is the proportion of non-inactivating current. A student t-test has been used to compare each mutant values with the control Ca_v2.3, and differences were considered statistically significant (marked by *) when P<0.05.

Supplementary Figure 1

The use of voltage-ramps does not distort current-voltage or AMF curves.

A. Current-voltage curves recorded using voltage-steps from -60 mV (or -80mV) mV to +60mV (or +70mV) on oocytes expressing $\text{Ca}_v2.3$ (or $\text{Ca}_v1.2$) in 10 mM Ba^{2+} (square) or 10 mM Ca^{2+} (circle) solutions. The superimposed smooth curves are the current traces recorded on the same oocyte during voltage-ramps of the same amplitude in Ba^{2+} (in red) or in Ca^{2+} (in blue). Thresholds, half-activation potentials, peaks and reversal potentials are almost identical, even for channel displaying Ca^{2+} dependent inactivation ($\text{Ca}_v1.2$). A shift of a few mV in V_{act} ($-2\pm 1\text{mV}$; $n=8$ for $\text{Ca}_v2.3$ in Ba10 for exemple) was sometimes noted between the two curves. This was in part due to the gating properties of the channels. The 10mV increment between the step potentials used to record the current-voltage curves may also bring a few mV of imprecision in the determination of the activation parameters. For our purposes, we considered this shift to be not significant. The differences in the other parameters (k and E_{rev}) were of the same amplitude ($0.1\pm 0.1\text{mV}$ and $3\pm 1\text{mV}$, respectively).

B. Averaged anomalous mole fraction effects obtained from current-voltage curves recorded on oocytes expressing $\text{Ca}_v1.2 + \alpha 2-\delta + \beta_{2a} \text{Ca}^{2+}$ channel subunits and using either voltage-ramps (-80/+80 mV at 2.5V/s, square) or voltage steps (400 ms duration, from -80mV to +80 mV in 10mV increment, circle). In both cases, the peaks of the current-voltage curves realized in the different Ba/Ca mole fraction solutions were used to trace the AMFE. Note the lack of significant differences between these two experimental procedures.

C. Averaged Ca to Ba permeability ratios for the different mutants channels used in this study calculated from current-voltage curves obtained with voltage-ramps or voltage-steps. In each case the ratios of the maxima of the current-voltage curves recorded in Ca^{2+} and in Ba^{2+} are shown.

Supplementary Figure 2.

A. Comparative analysis of the Ba/Ca permeability ratios and presence/absence of the AMFE between different LVA and HVA VGCC allow the identification of a specific permeation profile for divalent cation for each member of the LVA and HVA calcium channel families.

B. Amino acids alignment of the Ca_v2.3 VGCC residues with the corresponding pore region of the K_vAP channel. The EEEE and DCS loci are boxed; the residues of the P and S6 helices are visualized. Thr196 and Tyr199 used for the amino acids alignment are marked by an arrow.

Supplementary Figure 3. Mutations at the DCS locus do not affect channel gating.

A. Current-voltage curves obtained with the different Ca_v2.3 mutants co-expressed with $\alpha 2$ - δ and β_{2a} subunits in the presence of 10 mM Ba²⁺ (left) or Ca²⁺ (right). Ca_v2.3(TEQE), □; Ca_v2.3(DEEE), ○; Ca_v2.3(DSED), △; Ca_v2.3(DEQN), ▽; Ca_v2.3(TSQN)◇; Ca_v2.3(AAAA), ◁. See also supplementary tables II and III.

B. Isochronal inactivation curves (conditioning pulse of 2.5 s duration) obtained with the different Ca_v2.3 mutants co-expressed with $\alpha 2$ - δ and β_{2a} subunits in the presence of 10 mM Ba²⁺ (left) or Ca²⁺ (right). See also supplementary tables II and III. The only noticeable difference is the larger inactivation recorded with the Ca_v2.3(AAAA) mutant. This modification cannot explain the monotonic decrease of the AMF because (i) this occurred without any modification of the activation parameters and the inactivation kinetics and (ii) the Ca_v2.3(GGGG) which has the same potentials for half activation and inactivation than the wild type also displays monotonic AMF with smaller I_{Ca}/I_{Ba} ratio suggesting that change in inactivation and cation selectivity are not related.

C. Time course of inactivation of these mutant channels recorded in Ba²⁺ or Ca²⁺ solutions and quantified as the fractional current at 2.5 s (current amplitude at the end of the depolarization divided by the current amplitude at the peak). None of the small differences shown in this graph, between the mutant Ca_v2.3 channels and between Ba²⁺ and Ca²⁺ currents, is statistically significant. The Ca_v2.1(TEQE) is also shown and has clearly no Ca²⁺-dependent inactivation

Supplementary Figure 4. The EEEE locus also shapes the Ca/Ba permeation profile

A. Non-conserved residue between $\text{Ca}_v2.3$ and $\text{Ca}_v1.2$ ($\text{Ca}_v2.3$ (G₁₃₂₃F), reported to modify the EEEE locus decreases ICa/IBa (top) and AMFE (bottom) in a proportion expected from the change in experimentally-determined divalent cation binding energy(30) at the EEEE locus. Mutation of the other non-conserved residue between $\text{Ca}_v2.3$ and $\text{Ca}_v1.2$ ($\text{Ca}_v2.3$ (S₁₆₁₁C) has no effect as suspected from its extra-loci location. On the **top** are shown the Ca^{2+} channel currents recorded during the usual voltage-ramps under different $\text{Ba}^{2+}/\text{Ca}^{2+}$ mole fractions on the bottom, the corresponding current-mole fraction curve for each mutant ($\text{Ca}_v2.3$: \square ; $\text{Ca}_v2.3(\text{G}_{1323}\text{F})$: ∇ ; $\text{Ca}_v2.3(\text{S}_{1611}\text{C})$: \circ).

B. Left. Simulated ICa/IBa ratios calculated for decreasing free energies at the internal EEEE locus of the $\text{Ca}_v2.3$ channel. **Right.** Simulated currents-mole fraction curves with the decreasing EEEE ΔG° (bottom) depicted on the left. These curves were labeled $\text{Ca}_v2.3$, and $\text{Ca}_v2.3(\text{G}_{1311}\text{F})$, after comparison with original traces shown on A, and suspected variations in free energy at EEEE (30) loci due to mutation.