Review

Low Voltage Activated Calcium Channels: from Genes to Function

Ľ. Lacinová[1,2], N. Klugbauer[1] and F. Hofmann[1]

1 *Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Str. 29, 80802 München, Germany*
2 *on leave from Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 04 Bratislava, Slovakia*

Abstract. Cloning of three members of low-voltage-activated (LVA) calcium channel family, predominantly neuronal α_{1G} and α_{1I}, and ubiquitous α_{1H}, enabled to investigate directly their electrophysiological and pharmacological profile as well as their putative subunit composition. All the three channels are half-activated at membrane potential about −40 mV and half-inactivated at about −70 mV. Kinetics of α_{1G} and α_{1H} channels activation and inactivation are similar and faster than that of α_{1I} channel. All the three channels are blocked with high affinity by the organic blocker mibefradil. Another high affinity blocker is kurtoxin. Cloned LVA channels are relatively insensitive to antiepileptics, dihydropyridines and ω-conotoxins. Ni^{2+} is high affinity blocker of α_{1H} channel only. Amiloride inhibits the α_{1H} channel.

The subunit composition of LVA channel remains unclear. Out of known high-voltage-activated calcium channel subunits, $\alpha_2\delta$-2 and γ-5 subunits significantly and systematically modified activation and/or inactivation of the current. In contrast, $\alpha_2\delta$-1, $\alpha_2\delta$-3, γ-2 and γ-4 subunits failed to modulate the current or had only minor effects.

Key words: T-type calcium channel — α_1 subunit — Mibefradil — Antiepileptics

Introduction

Voltage-gated calcium channels are large transmembrane proteins that regulate the intracellular concentration of calcium ions. They are classified into two families according to the membrane potential at which they are activated: high (HVA) and low (LVA) voltage-activated channels (Carbone and Lux 1984; Nilius et al. 1985; Nowycky et al. 1985). The threshold membrane potential for activation of

Correspondence to: Ľ. Lacinová, Institut für Pharmakologie und Toxikologie der Technischen Universität München, Biedersteiner Str. 29, 80802 München, Germany. E-mail: lacinova@ipt.med.tu-muenchen.de

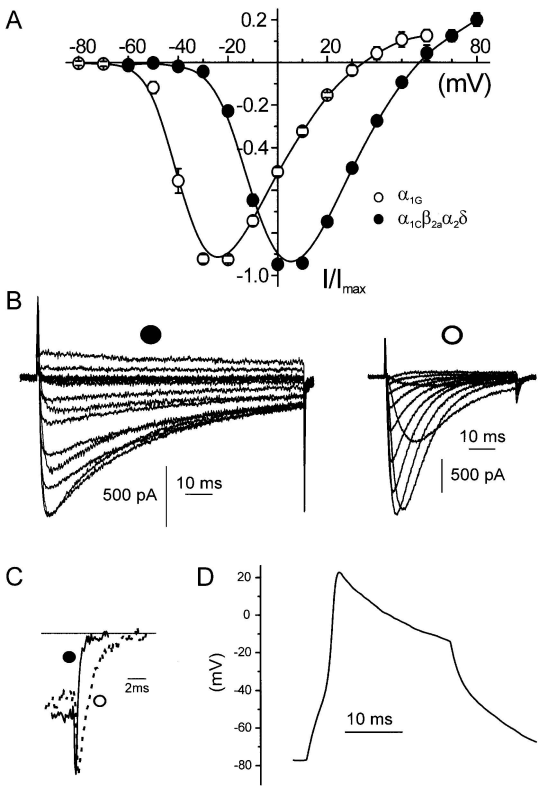

Figure 1. A. comparison of the voltage dependence of activation of high- and low-voltage activated (HVA and LVA) calcium channels. α_{1G} as a representative of LVA channels and $\alpha_{1C}\beta_{2a}\alpha_2\delta$ as a representative of HVA channels were transiently expressed in HEK 293 cells. Calcium current was measured with 20 mmol/l Ca^{2+} as the charge carrier. Current – voltage (I-V) relationships were normalized for better comparison. **B.** examples of calcium currents measured during (I-V) relation protocol in panel A. (○) – α_{1G} channel, (●) – $\alpha_{1C}\beta_{2a}\alpha_2\delta$ channel. **C.** examples of tail currents from the α_{1G} channel (○) and $\alpha_{1C}\beta_{2a}\alpha_2\delta$ channels (●) shown on extended time scale and scaled to the same amplitude to facilitate comparison of decay kinetics. **D.** upon expression of the α_{1G} channel in HEK 293 cells low threshold Ca^{2+} spikes could be activated by applying a current pulse.

macroscopic LVA calcium current is about −60 mV, which is below the threshold potential for action potential generation and is far more negative than for other voltage-gated calcium channels (Fig. 1 A). LVA channels open and inactivate very fast, but deactivate about 10 to 100 times slowlier than HVA calcium channels (Figs. 1 B and C). Single channel conductance of LVA channels is very low and is between 5 and 9 pS. For these reasons they are also called T-type: T for transient (fast inactivation) and tiny (small conductance). LVA channels can be detected in

various tissues such as heart, brain, dorsal root ganglia and adrenal gland. In the heart, T-type channels may contribute to the generation of the action potential in the sino-atrial node, to depolarization of the cell membrane and to the propagation of atrio-ventricular calcium action potential. The functional role of T-type channels in generating low-threshold spikes (Fig. 1 D) and rebound burst-firing has been demonstrated in neurons from the inferior olive, thalamus, hippocampus and neocortex (Huguenard 1996). In the adrenal gland, T-type channels are postulated to be involved in hormone secretion (Cohen et al. 1988). There is also evidence that genetic abnormalities in T-type channel genes give rise to absence epilepsy and cardiomyopathy (Sen and Smith 1994; Tsakiridou et al. 1995; Talley et al. 2000).

Cloning of low voltage-activated calcium channels

The cloning strategy used to identify the T-type calcium channels is an example of refined search of sequence databases using motifs common to the family of voltage gated calcium and sodium channels. Since the degree of homology between LVA and HVA calcium channels is rather low, standard techniques such as library screening or PCR amplification with degenerate primers are not applicable. The use of different search algorithms on mammalian expressed sequence-tagged cDNAs or on similar sequences of the nematode *Caenorhabditis elegans* led to the identification of several genes, three of which encode low voltage-activated calcium channels (Perez-Reyes et al. 1998; Cribbs et al. 1998; Lee et al. 1999a; Klugbauer et al. 1999; Williams et al. 1999). They have been named α_{1G}, α_{1H} and α_{1I}, or, according to a new nomenclature $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$, respectively (Fig. 2).

The phylogenetic relationship of calcium channels with putative ion channels from rat brain (rb21) and from the nematode *C. elegans* (C27F2, C11D2) are presented in Fig. 2. The homology between the 3 members of the LVA calcium channels is about 80%, which is comparable with that between members of the L-type and non-L-type calcium channels.

Predictions of higher order structures revealed that T-type channels – like HVA channels – contain four homologous repeats each consisting of six transmembrane segments. A comparison of the negatively charged residues in the pore loops shows that all T-type channels have a glutamate in repeats I and II and an aspartate in repeats III and IV (EEDD) whereas HVA channels have glutamates in all four repeats (EEEE). Although not proven so far, these differences may account for the altered ion selectivity of LVA and HVA channels and for the relative low unitary conductance of T-type channels. The positively charged residues of the HVA S4 voltage sensor are also conserved in LVA channels.

A fourth putative ion channel was identified recently. The rb21 cDNA predicts a protein that is structurally related to the superfamily of voltage gated sodium and calcium channels (Lee et al. 1999a). This putative channel did not conduct current in expression studies. It was speculated that the protein might have diverged from an ancestral four-domain channel before the divergence of calcium and potas-

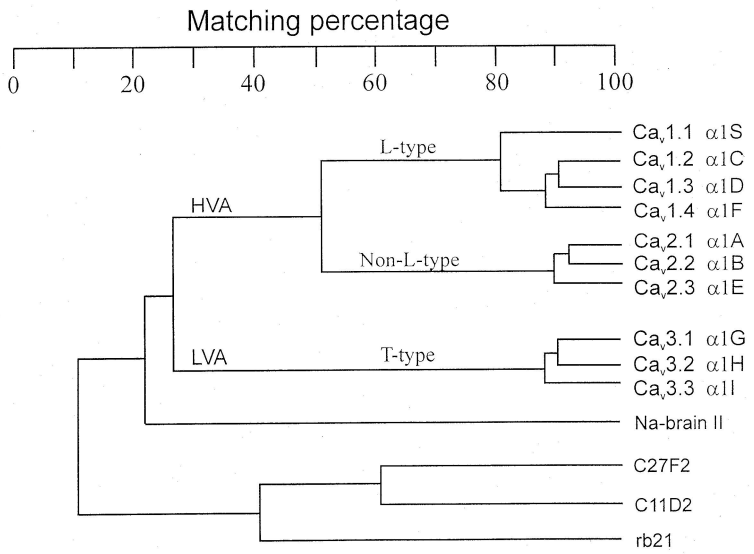

Figure 2. Phylogenetic comparison of all the known voltage-gated calcium channels with two putative channel proteins from *C. elegans* (C27F2 and C11D2) and the brain type II sodium channel. The amino acid alignment was constructed using the CLUSTAL program. Only regions encoding transmembrane segments were included in the alignment.

sium channels. Interestingly, this channel has a unique composition of negatively and positively charged residues in the pore loops of the four domains. Its EEKE composition is not found in calcium channels (either EEEE or EEDD) or sodium channels (DEKA). Site directed mutagenesis showed that the mutation of EIII to KIII changed the calcium selective channel into a monovalent cation-selective channel impermeable for divalent ions (Parent and Gopalakrishnan 1995).

Different splice variants of the α_{1G} channel have been reported for rat, mouse and human brain (Perez-Reyes et al. 1998; Klugbauer et al. 1999; Cribbs et al. 2000;

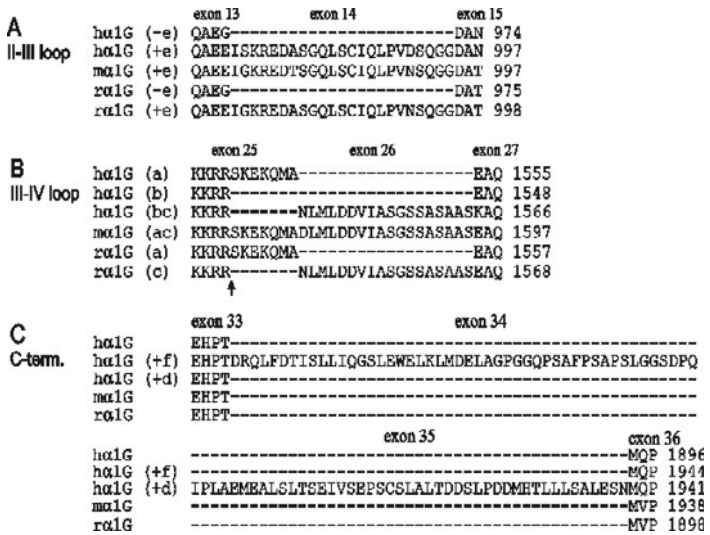

Figure 3. Alternative splicing events leading to various isoforms of the α_{1G} calcium channel. **A.** Alternate usage of exon 14 leads to two different isoforms in the loop between domains II and III. **B.** Splicing events that cause various isoforms in the loop between domains III and IV result from an alternate usage of exon 26 and an alternate 5′-splice donor site within exon 25 (indicated by the arrow). **C.** Alternate usage of either exon 34 or exon 35 or neither of them at the carboxy terminus of the α_{1G} calcium channel. The following sequences were included in the alignment: human hα_{1G}: accession number AF126966; mouse mα_{1G}: accession number AJ012569; rat rα_{1G}: accession number: AF027984. The numbers indicate the last amino acid.

Monteil et al. 2000a). Differentially spliced sequences are found in the intracellular loops connecting repeats II-III and III-IV and at the carboxy terminus (Fig. 3). The first alternatively spliced region is located immediately following IIS6 and is caused by insertion or skipping of an exon encoding 23 amino acid residues. This exon is not only present in the mouse brain, but also in cDNA isolated from human thalamus and cerebellum (Dubin et al. 2000; Monteil et al. 2000a). The second alternatively spliced region is located in the middle of the III–IV loop and results from the combination of two events that lead to four different transcripts. First, an alternative 3′splice donor site is used leading to seven additional amino acids. Second, there is an alternative use of an exon encoding 18 amino acid residues (Cribbs et al. 2000). The third alternatively spliced region is the carboxy terminus where 48 or 45 amino acids may be inserted (Monteil et al. 2000a). The occurrence of two carboxy terminal insertions is rather low. The functional significance of these splicing events is unclear at present.

The tissue distribution of T-type mRNA was analysed by Northern blot and *in situ* hybridizations. Transcripts of α_{1G} were identified in rat, human and mouse in the brain and at lower levels in heart (Perez-Reyes et al. 1998; Klugbauer et al.

1999). Transcripts of α_{1H} are expressed more ubiquituously. High concentrations of the transcripts were identified in human kidney, liver and heart, and lower levels were present in brain, placenta, lung, skeletal muscle and pancreas (Cribbs et al. 1998; Williams et al. 1999). α_{1I} mRNA of 10.5 kb was only found in the brain on a rat multiple tissue blot. However, smaller fragments were also detectable in the kidney, thymus and liver (Lee et al. 1999a).

In situ hybridization analysis of rat and mouse brain revealed that all the three mRNAs are differentially expressed. Transcripts of α_{1G} were found to be widely, but not uniformly, spread in mouse brain (Klugbauer et al. 1999). Significant amounts of α_{1G} mRNA were seen in the cerebellum, hippocampus, thalamus and olfactory bulb. Weaker signals are observed in the cerebral cortex and septal nuclei. In the cerebellum, expression is restricted to the Purkinje cell layer. In the hippocampus, the pyramidal cell layers had high levels of transcripts with CA1 and CA3 staining more strongly than CA2. A high expression is also observed in the granular and hilus regions of the dentate gyrus. Within the olfactory bulb, strong hybridisation signals were seen in the mitral, granular and glomerular cells.

In addition to the mRNA expression, Craig et al. (1999) presented a detailed analysis of the α_{1G} protein localization. Immunoreactivity for α_{1G} was comparable with the mRNA expression and was observed in both neuronal somata and dendrites in various neuroanatomical regions of the CNS. T-type calcium channels have been shown to promote low-threshold spike generation in cells of the inferior olive, habenular nuclei, certain cells of the pontine reticular formation and thalamic nuclei. In these regions, immunoreactivity paralleled the expression of α_{1G} mRNA. However, in other regions such as dentate granular cells only a weak immunoreactivity was found, although T-type currents have been described in these cells.

A comparative in situ hybridisation of all the three cloned T-type channels was performed by Talley et al. (1999) who found that expression of the three genes was largely complementary in the rat central and peripheral nervous system. Hybridization with α_{1G} specific riboprobes confirmed and refined the above mentioned results. α_{1H} riboprobes hybridised strongest to sensory ganglia, pituitary, dentate gyrus granule neurons and thalamic reticular neurons. The latter contained also α_{1I} mRNA. Expression was found to be high in the olfactory tubercules for α_{1I} and α_{1H}, and the subthalamic nucleus for α_{1I} and α_{1G}.

Permeability properties of T-type calcium channels

Instead of the highly conserved glutamates in all four pore regions, all the three cloned T-type calcium channels have glutamates in the pore regions I and II but aspartates in the pore regions III and IV. There is indirect evidence that the aspartates of the pore regions III and IV control the relative low unitary conductance. Expressed α_{1G} channel has a single-channel conductance of 7.5 pS (Perez-Reyes et al. 1998). For α_{1H} channel both slightly lower (5.3 pS ; Cribbs et al. 1998) and slightly higher values (9.1 pS; Williams et al. 1999) were reported. Expressed α_{1I}

channel has the highest single-channel conductance at 11.0 pS (Lee et al. 1999a; Monteil et al. 2000b). Unitary conductances reported for all the three channels are 3 times smaller compared to that of the L-channel. T-type channels, in contrast to the HVA channels, are not selective for Ba^{2+} over Ca^{2+}. Permeability ratios of expressed α_{1G} channel are $Ba^{2+}/Ca^{2+} \sim 0.96$ and $Sr^{2+}/Ca^{2+} \sim 1.35$ (Monteil et al. 2000a). Unlike any of the HVA calcium channels, T-type channels inactivate faster with Ba^{2+} than Ca^{2+} as charge carrier (Klugbauer et al.; 1999; Klöckner et al. 1999; Monteil et al. 2000a).

Regulation of T-type calcium channels by voltage

All the three cloned T-type calcium channels possess putative charged S4 segments. It is probable that the mechanism of their activation by voltage is similar to that of other members of the so-called S4 channels family, i.e., Na^+, K^+ and Ca^{2+} channels. The gating of T-type calcium channels contrasts in many aspects with that of L-type channels. In comparison with L-type channels, the voltage dependence of T-channel activation is shifted by 20-30 mV in the hyperpolarized direction, inactivation is rapid and not inherently voltage-dependent and deactivation is slow.

Activation

Parameters of voltage-dependent activation (Table 1) are dependent on the concentration of the charge carrier used but are not influenced by choice of Ba^{2+} or Ca^{2+} ions (Klugbauer et al. 1999; Klöckner et al. 1999). The membrane potential for half-maximal current peak varies between approximately −45 mV at low charge carrier concentrations of 1–2 mmol/l (Perez-Reyes et al. 1998; Kozlov et al. 1999; Klöckner et al. 1999) and approximately −25 mV for high charge carrier concentrations of 20–40 mmol/l (Klugbauer et al. 1999; Williams et al. 1999; Lacinová et al. 1999). These values are common for all the three channels, α_{1G}, α_{1H} and α_{1I}. Detailed analysis of tail current-voltage relations revealed two components of current activation in α_{1G} and α_{1H} channels. For the α_{1G} channel, potentials for half-maximal activation were −41.8 mV and −14.7 mV in 2 mmol/l Ca^{2+} with the corresponding slope factors of 5.1 and 11.9 (Monteil et al. 2000a). For the α_{1H} channel, potentials for half-maximal activation were −25.1 mV and +25.5 mV and the corresponding slope factors were 7.5 and 14.7 in 15 mmol/l Ba^{2+} (Williams et al. 1999).

The time course of current activation could be described by a single time constant which decreases sharply with the increasing amplitude of the depolarising pulse. In the case of the α_{1G} and α_{1H} channels, this time constant varies from 8–10 ms just above the activation threshold to hundreds of microseconds for pulse amplitudes positive relative to the peak of the current-voltage relationship (Perez-Reyes et al. 1998; Klugbauer et al. 1999; Williams et al. 1999; Klöckner et al. 1999). In the case of the α_{1I} channel, the activation time constant decreases from approximately 50 to 5 ms with the increasing pulse amplitude when channels are expressed in mammalian HEK 293 cells (Lee et al. 1999a; Kozlov et al. 1999;

Klöckner et al. 1999). This time constant is two times slowlier when the channel is expressed in *Xenopus* oocytes (Lee et al. 1999a). The activation time constants of the α_{1G} and α_{1H} channels are independent of the expression system (Perez-Reyes et al. 1998; Klugbauer et al. 1999; Williams et al. 1999).

Inactivation and recovery from inactivation

The inactivation and recovery from inactivation of T-type calcium channels is much faster than in any HVA calcium channel. The time course of the current decay during a single depolarising pulse may be fitted by a single exponential (Table 2). Just above the activation threshold this time constant is about 50 ms for expressed α_{1G} and α_{1H} channels. With the increasing amplitude of the depolarising pulse

Table 1. Voltage dependent activation of expressed $\alpha 1$ subunits of LVA calcium channels. $V_{0.5}$ and k represent the half-maximal activation voltage and activation slopes, respectively, obtained from a fit of experimental data by the Boltzmann function. τ is the time constant of current activation; the values left and right to the arrow were calculated for traces obtained by depolarising pulses just positive to the activation threshold and to the peak of the current-voltage relation, respectively. In some of the cited articles only the latter value was reported.

Channel	Charge carrier	$V_{0.5}$ (mV)	k (mV)	τ (ms)	Expression system
α_{1G}	2 mmol/l $Ba^{2+(1)}$	−46.5	6.6		*X.* oocytes
	10 mmol/l $Ba^{2+(1)}$	−38.0		5 → 1	*X.* oocytes
	40 mmol/l $Ba^{2+(1)}$	−21.0			*X.* oocytes
	20 mmol/l $Ba^{2+(8)}$	−28.4	4.0	8 → 0.5	HEK 293
	20 mmol/l $Ca^{2+(3)}$			10 → 0.5	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$	−45.5		5 → 2	HEK 293
	2 mmol/l $Ca^{2+(9)}$	−51.2	4.2		HEK 293
α_{1H}	10 mmol/l $Ba^{2+(2)}$	−44.0	7.2	12 → 2	HEK 293
	15 mmol/l $Ba^{2+(5)}$	−25.1		→ 3	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$	−45.8		10 → 3	HEK 293
	10 mmol/l $Ca^{2+(7)}$			12 → 2	HEK 293
	10 mmol/l $Ba^{2+(7)}$			8 → 1.5	HEK 293
α_{1I}	10 mmol/l $Ba^{2+(4)}$	−24.7	8.1	35 → 4	HEK 293
	10 mmol/l $Ba^{2+(4)}$	∼ −25	∼ 8	70 → 8	*X.* oocytes
	2 mmol/l $Ca^{2+(4)}$	−45.0	7.6	→ 4	HEK 293
	2.5 mmol/l $Ca^{2+(6)}$			50 → 5	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$			25 → 7	HEK 293
	2 mmol/l $Ca^{2+(9)}$	−40.6	5.6		HEK 293

(1) – Perez-Reyes et al. 1998; (2) – Cribbs et al. 1998; (3) – Klugbauer et al. 1999; (4) – Lee et al. 1999a; (5) – Williams et al. 1999; (6) – Kozlov et al. 1999; (7) – Klöckner et al. 1999; (8) – Lacinová et al. 1999; (9) – Monteil et al. 2000b

Table 2. Voltage dependent inactivation of expressed $\alpha 1$ subunits of LVA calcium channels. $V_{0.5}$ and k represent the half-maximal inactivation voltage and inactivation slopes, respectively, obtained from a fit of experimental data by the Boltzmann function. τ is the time constant of current inactivation; the values left and right to the arrow were calculated for traces obtained by depolarising pulses just positive to the activation threshold and to the peak of the current-voltage relation, respectively. In some of the cited articles only the latter value was reported.

Channel	Charge carrier	$V_{0.5}$ (mV)	k (mV)	τ (ms)	Expression system
α_{1G}	40 mmol/l $Ba^{2+(1)}$	−50.0	3.2	50 → 10	*X.* oocytes
	20 mmol/l $Ba^{2+(3)}$	−57.3	5.6	25 → 10	HEK 293
	20 mmol/l $Ca^{2+(3)}$	−55.0	4.9	28 → 15	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$	−72.8	4.1	50 → 15	HEK 293
	2.5 mmol/l $Ca^{2+(6)}$			50 → 10	HEK 293
	2 mmol/l $Ca^{2+(9)}$	−74.7	5.3	20 → 14	HEK 293
α_{1H}	10 mmol/l $Ba^{2+(2)}$	−75.3	7.8	60 → 20	HEK 293
	15 mmol/l $Ba^{2+(5)}$	−63.2		→ 17	HEK 293
	2.5 mmol/l $Ca^{2+(6)}$			50 → 10	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$	−70.0	3.7	60 → 20	HEK 293
	10 mmol/l $Ca^{2+(7)}$			60 → 20	HEK 293
	10 mmol/l $Ba^{2+(7)}$			60 → 10	HEK 293
α_{1I}	10 mmol/l $Ba^{2+(4)}$	−68.3	6.3	170 → 55	HEK 293
	10 mmol/l $Ba^{2+(4)}$	−57.7		slowlier than in HEK 293	*X.* oocytes
	2 mmol/l $Ca^{2+(4)}$	−77.1	6.0	→ 75	HEK 293
	2.5 mmol/l $Ca^{2+(6)}$			200 → 150	HEK 293
	1.25 mmol/l $Ca^{2+(7)}$	−72.5	4.6	230 → 100	HEK 293
	2 mmol/l $Ca^{2+(9)}$	−68.9	5.6	273 → 89	HEK 293

(1) – Perez-Reyes et al. 1998; (2) – Cribbs et al. 1998; (3) – Klugbauer et al. 1999; (4) – Lee et al. 1999a; (5) – Williams et al. 1999; (6) – Kozlov et al. 1999; (7) – Klöckner et al. 1999; (8) – Lacinová et al. 1999; (9) – Monteil et al. 2000b

the current decay became faster and its time constant saturated at a virtually voltage independent value of 10–15 ms (Perez-Reyes et al. 1998; Cribbs et al. 1998; Klugbauer et al. 1999; Williams et al. 1999; Kozlov et al. 1999; Klöckner et al. 1999). This time constant is independent of the charge carrier concentration and of the expression system. Interestingly, both α_{1G} and α_{1H} channels inactivate faster with Ba^{2+} than Ca^{2+} as charge carrier (Klugbauer et al. 1999; Klöckner et al. 1999; Monteil et al. 2000a). In spite of the fast inactivation, there is a sustained current component corresponding to about 1–2% of the non-inactivated channels (Serrano et al. 1999). Recovery from inactivation by a 5 s long pulse to 0 mV is

fast and monoexponential with a time constant of 200 ms at a membrane potential of −100 mV (Klugbauer et al. 1999). When 1 s long inactivating pulse was used, in addition to the fast time constant in the range of ≈ 100 ms a slow time constant in the range of ≈ 1 s was identified in α_{1G} and α_{1H} channels (Satin and Cribbs, 2000). The relative amplitude of the slow time constant was about 20% for the α_{1G} channel and about 80% for the α_{1H} channel. The slowlier apparent recovery from inactivation of the α_{1H} channel seems to be the only significant difference in the electrophysiological profile of the α_{1G} and α_{1H} channel isoforms. Serrano et al. (1999) suggested a model in which the α_{1G} channel inactivates from any of the four closed states and an open state. The rate constants of channel inactivation and recovery in this model are state- but not voltage-dependent. Experimental observations are in accordance with the model.

The inactivation properties of the α_{1I} channel differ from those of the α_{1G} and α_{1H} channels. When expressed in HEK 293 cells, the channel inactivates much slowlier with time constants ranging from about 200 ms at depolarising pulses above the activation threshold to 50–100 ms at more positive depolarising pulses (Lee et al. 1999a; Kozlov et al. 1999; Klöckner et al. 1999; Monteil et al. 2000b). The inactivation rate is even slowlier when the channel is expressed in *Xenopus* oocytes (Lee et al. 1999a). Recovery from inactivation of the α_{1I} channel is monoexponential with the time constant of 297 ms (Monteil et al. 2000b).

Deactivation

Deactivation of T-type calcium channels is very slow in comparison with HVA calcium channels. Tail currents may be fitted by a single exponential. At extremely hyperpolarized membrane potentials below −100 mV, the time constant of tail current decay reaches a voltage-independent value of 1–2 ms for α_{1G} and α_{1H} channels. At membrane potentials positive to −100 mV, this time constant increases non-linearly (Serrano et al. 1999) and reaches a value 10–12 ms at −40 mV (Perez-Reyes et al. 1998; Cribbs et al. 1998; Klugbauer et al. 1999). In other words, current decay and current inactivation α_{1G} and α_{1H} channels converge to the same voltage-independent rate. The deactivation kinetics of α_{1G} channel is independent of the charge carrier (Ba^{2+}/Ca^{2+}; Klugbauer et al. 1999). The expressed α_{1I} channel deactivates faster with a time constant ranging from 0.3–0.5 ms at extremely hyperpolarized membrane potentials to 2 ms at membrane potential of −40 mV (Kozlov et al. 1999; Klöckner et al. 1999; Monteil et al. 2000b).

Pharmacology of T-type calcium channels

The cloning of the family of LVA calcium channels $\alpha 1$ subunits enabled studies of the pharmacology of T-type calcium channels. In native tissues, T-type currents are masked to a considerable extent by HVA calcium currents and have to be dissected using pharmacological and/or biophysical techniques. Published results have shown a considerable variability (Huguenard 1996) which has been attributed to the putative existence of multiple channel types. This hypothesis was confirmed

Table 3. Inhibition of expressed LVA channels by inorganic ions.

Ion	Channel	Charge carrier	Effect	Reference
Ni^{2+}	α_{1G}	10 mmol/l Ba^{2+}	IC_{50} = 250 μM	Lee et al. 1999b
		20 mmol/l Ba^{2+}	IC_{50} = 470 μM	Lacinová et al. 2000
		20 mmol/l Ca^{2+}	IC_{50} = 1130 μM	Lacinová et al. 2000
		2 mmol/l Ca^{2+}	IC_{50} = 133 μM	Monteil et al. 2000b
	α_{1H}	15 mmol/l Ba^{2+}	IC_{50} = 6.6 μM	Williams et al. 1999
		10 mmol/l Ba^{2+}	IC_{50} = 12 μM	Lee et al. 1999b
		5 mmol/l Ca^{2+}	IC_{50} (1) = 1.9 μM	Perchenet et al. 2000
			IC_{50} (2) = 1350 μM	
	α_{1I}	10 mmol/l Ba^{2+}	IC_{50} = 216 μM	Lee et al. 1999b
		2 mmol/l Ca^{2+}	IC_{50} = 184 μM	Monteil et al. 2000b
Cd^{2+}	α_{1G}	20 mmol/l Ba^{2+}	IC_{50} = 162 μM	Lacinová et al. 2000
		20 mmol/l Ca^{2+}	IC_{50} = 658 μM	Lacinová et al. 2000
	α_{1H}	15 mmol/l Ba^{2+}	IC_{50} = 104 μM	Williams et al. 1999
		5 mmol/l Ca^{2+}	IC_{50} = 218 μM	Perchenet et al. 2000

by the isolation of three genes, α_{1G}, α_{1H} and α_{1I}. Initial studies (Williams et al. 1999; Lacinová et al. 2000, Perchenet et al. 2000) revealed considerable differences between the α_{1G} and α_{1H} channels. The α_{1H} channel appears to be more sensitive than the α_{1G} channel to several T-type channel blockers characterized on the native channels. The pharmacology of the α_{1I} channel has not been characterized yet.

Inorganic cations

The high sensitivity of native T-type calcium current to block by Ni^{2+} was considered to be one of the characteristics of this channel. However, IC_{50} values observed in numerous native tissues varied between 30 μM and 780 μM (reviewed by Huguenard 1996). Experiments with recombinant channels revealed that expressed α_{1G} and α_{1I} channels have a low affinity to Ni^{2+} (see Table 3). A high affinity block by Ni^{2+} was found only with the expressed α_{1H} channel. More detailed analysis showed that this apparent high affinity block may actually consist of high and low affinity sites (see Table 3). This observation is supported by findings of Lee at al. (1999b) that the Hill coefficient for inhibition of α_{1H} channel by Ni^{2+} is significantly smaller than 1. The interaction of Ni^{2+} with all the three cloned T-type calcium channels is complex. The ion shifts the voltage dependence of current activation towards more positive membrane voltages, increases the slope of the voltage dependence of current activation, and accelerates channel deactivation of the α_{1G} channel (Lacinová et al. 2000). Ni^{2+} slows down the inactivation time course of the α_{1H} channel (Lee et al. 1999b). Block by Ni^{2+} is voltage-dependent in all the

Table 4. Effect of organic blockers on α_{1G} and α_{1H} channels.

Drug	α_{1G}	α_{1H}	Reference
Mibefradil	$IC_{50} = 0.39$ μmol/l		Klugbauer et al. 1999
Mibefradil		$IC_{50} \approx 1.2$ μmol/l	Cribs et al. 1998; Williams et al. 1999; Perchenet et al. 2000
Kurtoxin	$K_D = 15$ nmol/l	$K_D = 61$ nmol/l	Chuang et al. 1998
Arachidonic acid		$IC_{50} \leq 10$ μmol/l	Zhang et al. 2000
Amlodipine		$IC_{50} = 30.9$ μmol/l	Perchenet et al. 2000
Amiloride		$IC_{50} = 167$ μmol/l	Williams et al. 1999
Amiloride	$IC_{50} \geq 5$ mmol/l		Lacinová et al. 2000
Phenytoin	$IC_{50} = 74$ μmol/l		Lacinová et al. 2000
Ethosuximide		$IC_{50} < 300$ μmol/l	Williams et al. 1999
Ethosuximide	$IC_{50} > 3$ mmol/l		Lacinová et al. 200
Valproate	max. block 10% at 1 mmol/l		Lacinová et al. 2000
Verapamil		$IC_{50} > 1$ μmol/l	Williams et al. 1999
Nimodipine		$IC_{50} \approx 10$ μmol/l	Williams et al. 1999
Isradipine	$IC_{50} \gg 1$ μmol/l		Lacinová et al. 2000
Nifedipine	$IC_{50} \gg 10$ μmol/l		Lacinová et al. 2000
Bay K 8644		10 μmol/l min. effect	Williams et al. 1999
Bay K 8644	1 μmol/l min. effect		Lacinová et al. 2000
TTX	10 μmol/l no effect		Lacinová et al. 2000
TTX		30 μmol/l no effect	Perchenet et al. 2000
ω-Aga-IVA		60 nmol/l no effect	Perchenet et al. 2000
ω-CTx-MVIIC		1 μmol/l no effect	Perchenet et al. 2000; Williams et al. 1999
ω-CgTxGVIA		1 μmol/l no effect	Perchenet et al. 2000; Williams et al. 1999

three expressed channels and may be relieved at very positive membrane voltages around +100 mV (Lee et al. 1999b).

Another divalent cation channel blocker, Cd^{2+}, blocks α_{1H} slightly more effectively than α_{1G} (Table 3). In addition to the block of the current amplitude, Cd^{2+} accelerates the time constant of deactivation of the expressed α_{1G} channel (Lacinová et al. 2000).

Organic blockers

Selective inhibition of T-type calcium channels may have clinical implications in cardiovascular diseases (Katz 1999) and some forms of epilepsy (Macdonald and Kelly 1995). An overview of the effects of organic channel blockers on expressed LVA channels together with the corresponding references is given in Table 4. For native T-type calcium channels, the only organic blocker effective at submicromolar concentrations is mibefradil (for review see Clozel et al. 1997). Mibefradil also

inhibits expressed α_{1G}, α_{1H} and α_{1I} channels in nanomolar concentrations. The lowest affinity has the α_{1I} channel with IC_{50} of 2.3 μmol/l (Monteil et al. 2000b). The availability of the cloned α_{1G} channel enabled identification of the scorpion toxin kurtoxin which has a high affinity for both α_{1G} and α_{1H} channels. The expressed α_{1G} channel has a low sensitivity to the antiepileptic drugs valproate and ethosuximide and is moderately sensitive to phenytoin. The α_{1H} channel is sensitive to ethosuximide, amlodipine and amiloride. In contrast to several reports on native T-type calcium channels (for references see Lacinová et al. 2000), both channels are resistant to both agonist and antagonist dihydropyridines. The α_{1H} channel is also resistant to spider toxins known to inhibit neuronal HVA calcium chanels. A TTX-sensitive low-voltage activated Ca^{2+} current has been identified in cardiac and neuronal preparations (Aggarwal et al. 1997; Balke et al. 1999). This current was insensitive to low concentrations of Ni^{2+} and its kinetics resembled that of a T-type calcium channel. Both α_{1G} and α_{1H} channels are insensitive to TTX and therefore cannot contribute to this type of calcium conductance in native tissues. The amplitude of the α_{1H} current is inhibited by low concentrations of arachidonic acid. This modulation may have pathophysiological significance. External acidification from pH 8.2 to 5.5 modulates the activity of the α_{1H} channel in a complex way (Delisle and Satin 2000). At pH 5.5, the current amplitude is inhibited and the voltage dependences of both channel activation and inactivation are shifted in the depolarizing direction. Activation gating is slowed down while deactivation is accelerated. Paradoxically, acidification increases the macroscopic slope conductance. Altogether, acidification attenuates the activity of the α_{1H} channel and might contribute to the protection against abnormal rhythm generation during ischemia.

Subunit composition of T-type calcium channels

The kinetics of expressed α_{1G} and α_{1H} channels activation, inactivation and deactivation mimic the kinetics observed in native T-type calcium channels (for review see Huguenard 1996). It is therefore possible that these LVA channels may consist of a single α_1 subunit protein which contains the voltage-sensor, the selectivity filter, the ion-conducting pore and also the binding sites for the T-type channel blockers mibefradil and kurtoxin. Several other findings support this hypothesis. The amino acid sequence of all the three cloned α_1 subunits lacks the α subunit interaction domain (AID) identified in α_1 subunits of HVA channels. The AID domain was shown to be necessary for the interaction between HVA channel α_1 subunit with β subunit (for review see Hofmann et al. 1999). Elimination of the four known β subunits by transfection of nodus ganglion neurons (Lambert et al. 1997) or mammalian neuronal NG108-15 cells (Leuranger et al. 1998) with antisense oligonucleotides, or overexpression of the neuronal β_{2a} subunit (Wyatt et al. 1998) did not affect the size or voltage-dependence of the native T-type current. Coexpression of the α_{1G} subunit with the $\alpha_2\delta$-1 or $\alpha_2\delta$-3 subunit did not (Lacinová

et al. 1999) or only minimally modulate (Dolphin et al. 1999) the T-type current. Some expression studies support the notion that the $\alpha_2\delta$-2 (Hobom et al. 2000) and the γ_5 subunit (Klugbauer et al. 2000) may moderately modulate the current through the α_{1G} channel.

Activation and inactivation kinetics of the expressed α_{1I} channel are slowlier than the kinetics of the native T-type current (Lee et al. 1999a; Kozlov et al. 1999; Klöckner et al. 1999). This observation together with the reported dependence of the channel kinetics on the expression system (Lee et al. 1999a) suggests that this LVA channel is regulated by an auxiliary subunit and/or other factors endogenously present either in HEK 293 cells or in *Xenopus* oocytes. Alternatively, the predominant α_{1I} subunit may have another carboxy terminus because of the extraordinary short C-terminus and insertion of repetitive sequence that is not present in the human sequence (Dunham et al. 1999).

The effects of the known auxiliary subunits on LVA channel pharmacology have not been described until recently. Currently, we can not rule out that the native T-type calcium channels consist of an α_1 subunit and so far unidentified auxiliary subunits.

In conclusion, heterologous expression of the members of the LVA channels family enabled to characterize their basic electrophysiological and pharmacological profile unbiased by the necessity to block other ion channels, which are usually expressed in native cells with higher densities than LVA channels. Further investigation will be directed to reveal structural determinants of intrinsic and extrinsic channel regulations.

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