

# Voltage-Dependent Calcium Channels

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**Voltage-dependent calcium channels are key mediators in fast signal transduction in excitable and non-excitable cells. They are formed by up to 5 subunits, with the main  $\alpha_1$  subunit and  $\alpha_2\delta$ ,  $\beta$  and  $\gamma$  as auxiliary subunits, modulating channel expression and current kinetics. Molecular biology studies have identified 10 different genes each coding for an  $\alpha_1$  subunit. They are divided into three subfamilies,  $Ca_v1$ ,  $Ca_v2$  and  $Ca_v3$ , according to their sequence similarities. The  $Ca_v1$  isoforms correspond to the dihydropyridine-sensitive L-type channels, the  $Ca_v2$  isoforms represent the neuronal currents (N-, P/Q-, R-), sensitive to spider and cone snail toxins, and the  $Ca_v3$  isoforms form the so called T-type channels, which are insensitive against either the "typical" L-type blockers or the organic non-L-type inhibitors.**

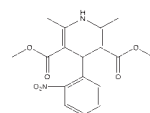
## Introduction

Voltage-dependent calcium channels (VDCC) represent the main calcium entry pathways in many cell types and regulate intracellular processes such as cell contraction<sup>1</sup>, gene transcription<sup>2</sup>, synaptic plasticity<sup>3</sup>, and hormone secretion<sup>4</sup> (for review see<sup>5</sup>). They were first identified in 1953 by Fatt and Katz in crustacean muscle<sup>6</sup>. Mammalian calcium channels were purified from skeletal muscle after drug labelling analysis with dihydropyridine (DHP), phenylalkylamine (PAA) and benzothiazepine (BTZ) radioligands in the 80's<sup>7-11</sup>. Later calcium channels were also recognized in and cloned from cardiac and smooth muscle, and subsequently in most excitable (and also in non-excitable) tissues<sup>12-17</sup>. Initially, electrophysiological and pharmacological criteria were used to classify the channels according to their current properties. Thus, high-voltage-activate (HVA) channels were distinguished from low-voltage-activated (LVA) channels. The HVA channels require membrane voltages positive to -30 mV for activation. Their current is large and long lasting. Therefore, they were also termed L-type channels. Typically, L-type channels are blocked by organic L-type channel antagonists like the Dihydropyridines, Phenylalkylamines and Benzothiazepines. In contrast, the LVA channels are activated at very negative voltages around -70 mV. The current activates and inactivates quickly (transient) and the single channel conductance is small (tiny). Therefore, they were also termed T-type channels. LVA channels can be detected in various tissues such as heart, brain, dorsal root ganglia and hormonal tissues. In the heart, they contribute to the pacemaker function of the sinoatrial node and the propagation of the action-potentials. In the brain, abnormalities in T-type channels give rise to epileptic episodes. T-channels are insensitive to "typical" calcium channel blockers and until now lack highly specific activators and inhibitors. In neurons another class of HVA calcium channels was found

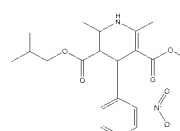
which was also insensitive to L-type channel blockers<sup>14, 15</sup>. Their single-channel conductance was described to be between those of T-type and L-type channels. This neuronal non-L-type channels were termed N-type (neuronal, neither L- nor T-type)<sup>18, 19</sup>. Later, they were further divided in N-, P-, Q- and R-type channels according to their sensitivity versus different peptide toxins isolated from cone snails and spiders. In many cell types, P- and Q-type current components cannot be adequately separated and many researchers in the field have adopted the terminology P/Q-type current when referring to either component. N-type channels are typically inhibited by  $\omega$ -Conotoxin GVIA (Figure 1), whereas P/Q- and R-type channels are antagonized by  $\omega$ -Agatoxin IVA (Figure 1) and SNX 482 (Figure 1), respectively (see also Table 2). For the interested reader excellent reviews give a more detailed survey on structure and function of the calcium channels<sup>20-25</sup>.

## DHPs

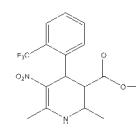
### Antagonists



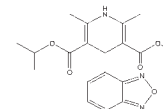
Nifedipine (BG0268)



Nisoldipine (BG0270)

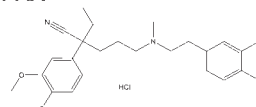


(RS)-Bay K 8644 (BN0100)



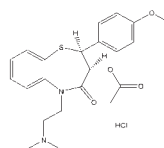
Isradipine (BG0371)

## PAA



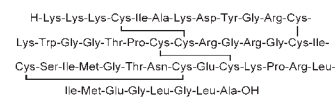
Verapamil hydrochloride (BG0353)

## BTZ

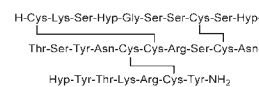


Diltiazem hydrochloride (BG0366)

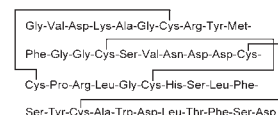
## Agonist



$\omega$ -Agatoxin IVA (BP0022)



$\omega$ -Conotoxin GVIA (BP0079)



SNX 482 (BP0376)

**Figure 1.** Structure of selected voltage-dependent calcium channel compounds available from BIOTREND (with catalogue numbers)

## Subunit composition and function

Calcium channels are heterooligomeric protein complexes of up to 5 subunits. The main  $\alpha_1$  subunit (212–250 kDa) forms the pore, expresses the selectivity filter and the voltage-sensor and carries the binding sites for all known calcium channel agonists and antagonists. It is composed of four homologous repeats (I through IV) that contain six transmembrane alpha-helical segments (S1–S6), each. The pore is formed by the linker between segment 5 and 6 of each domain, which loops back from the extracellular part of the membrane (SS1–SS2; pore loop; P-region)<sup>26</sup>. The pore loop determines the ion conductance and selectivity. A glutamate residue is found at corresponding positions in each of the SS1–SS2 regions of repeats I, II, III and IV in all HVA calcium channels. Together, they form a high-affinity calcium-binding site. In LVA channels aspartates instead of glutamates are expressed in repeats III and IV. This is the cause for the different ion selectivity of the channels. In line with this, it has been shown that replacing a lysine in repeat III and/or an alanine in repeat IV of a rat sodium channel by glutamic acid, which occurs at the equivalent positions in calcium channels, alter ion-selection properties of the sodium channel to resemble those of calcium channels<sup>27</sup>. Another important structure is formed by the S4-segments, which carry a positively charged amino acid residue (arginine or lysine) at every third position and thus form the voltage-sensor. On opening or closing the voltage-sensor carries gating charges through the membrane. How S4 moves at gating is debated: either S4 moves in a helical screw or as a paddle sweeping through the lipid bilayer in response to voltage changes<sup>28, 29</sup>. The regions involved in activation as well as calcium- and voltage-dependent inactivation are also located at the  $\alpha_1$  subunit. Furthermore, the  $\alpha_1$  subunit contains different putative phosphorylation sites for different protein kinases like PKA (protein kinase A), PKC (protein kinase C) and CaMKII (calmodulin-dependent protein kinase type II) as well as G-protein interaction sites. Usually, the  $\alpha_1$  subunit is associated with the auxiliary  $\beta$ , the  $\alpha_2\delta$  and (only in a few tissues) the  $\gamma$  subunit. The interaction site of the  $\beta$  subunit with the  $\alpha_1$  subunit is located at the cytoplasmic linker between domain I and II of the  $\alpha_1$  subunit<sup>30</sup>. The 18 amino acid motif is called the alpha subunit interaction domain (AID). The  $\beta$  subunits are intracellular located small proteins (50–72 kDa)<sup>31</sup>. They are usually associated with HVA  $\alpha_1$  subunits. Coexpression of  $\beta$  subunits with LVA channels has not been demonstrated. Heterologous expression of  $\alpha_1$  and  $\beta$  subunits increases the peak current, most likely by increasing the number of functional channels at the cytoplasmic membrane and by facilitating the channel opening<sup>32–40</sup>. The  $\alpha_2$  and the  $\delta$  subunit are encoded in the same gene<sup>41</sup>. Biochemical studies showed that the  $\alpha_2$  subunit is mainly extracellular, and is tethered via disulfide bonds to the  $\delta$  subunit that crosses the plasma membrane<sup>42</sup>. Coexpression of the  $\alpha_2\delta$  subunit with different  $\alpha_1$  and  $\delta$  subunits increases the current density and changes channel kinetics<sup>32, 43–45</sup>. The  $\gamma$  subunits are predominantly expressed in the skeletal muscle, but have also been detected in retina, brain and other tissues<sup>46–49</sup>. The precise function of the  $\gamma$  subunits is unresolved. Expression studies suggest an ability to normalize calcium currents to those resembling native channels<sup>46</sup>. Later, it was shown that the  $\gamma$ -1 subunit of the skeletal muscle L-type calcium channel is an endogenous calcium channel antagonist<sup>50</sup>. Expression of the  $\alpha_1$  subunit is sufficient to form the pore through which calcium enters the cell

and the interaction sites for calcium channel blockers and agonists<sup>32, 33, 51–53</sup>. Coexpression of  $\text{Ca}_v1.2$   $\beta$  subunits influences calcium channel ligand binding sites<sup>54</sup>. This is due to an increased number of channels and/or differential effects on channel inactivation caused by the coexpressed  $\beta$  subunit<sup>55, 56</sup>.

## Current classification according to calcium channel genes

Until now, ten different genes coding for  $\alpha_1$  subunits have been identified in vertebrates. Four of them represent L-type calcium channels. The first to be identified was the skeletal muscle  $\alpha_{1S}$  subunit, followed by the cardiac and smooth muscle  $\alpha_{1C}$  subunit<sup>8, 12, 13</sup>.  $\alpha_{1D}$  was cloned later from pancreatic  $\beta$ -cells and neurons, respectively<sup>57, 58</sup> and was termed the neuroendocrine calcium channel type. Recently, an additional L-type channel was identified which is only expressed in the retina and was called  $\alpha_{1F}$ <sup>59, 60</sup>. Three genes,  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1E}$ , code for the neuronal calcium channels. The  $\alpha_{1A}$  subunit corresponds to the P/Q-type current<sup>61, 62</sup>, whereas the N-type current was matched by  $\alpha_{1B}$ <sup>63, 64</sup>.  $\alpha_{1E}$  was first characterized as a T-type calcium channel because of its rapid activation and inactivation kinetics and its voltage dependence<sup>65</sup>. Later it was demonstrated that it more likely resembles the R-type channel<sup>63, 66</sup>. Instead  $\alpha_{1G}$ ,  $\alpha_{1H}$  and  $\alpha_{1I}$  were identified as members of the T-type calcium channel family<sup>67–70</sup>. Various names arose from different laboratories exacerbating the comparability of results. Therefore, an alphabetical nomenclature was introduced in the 1990's, with  $\alpha_{1S}$  representing the original skeletal muscle isoform,  $\alpha_{1C}$  the cardiac channel and  $\alpha_{1A}$  though  $\alpha_{1I}$  for those discovered subsequently (Table 1)<sup>71</sup>. Since 2000 a rational nomenclature adopted from the potassium channels is used<sup>72</sup> which was approved by the NC-IUPHAR subcommittee on calcium channels. The channels were named using the chemical symbol of the principal permeating ion with the principal physiological regulator indicated as a subscript ( $\text{Ca}_v$ : calcium, voltage)<sup>73</sup>. The different subfamilies were named with numerical identifiers followed by the number of the  $\alpha_1$  subunit in order of their discovery. Thus, the L-type channels are named  $\text{Ca}_v1.1$  through  $\text{Ca}_v1.4$  corresponding to their main  $\alpha_{1S}$ ,  $\alpha_{1C}$ ,  $\alpha_{1D}$  and  $\alpha_{1F}$  subunit. The  $\text{Ca}_v2$  subfamily ( $\text{Ca}_v2.1$ ,  $\text{Ca}_v2.2$ ,  $\text{Ca}_v2.3$ ) includes channels containing the  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1E}$  subunit. The T-types are named  $\text{Ca}_v3.1$  through  $\text{Ca}_v3.3$ , corresponding to  $\alpha_{1G}$ ,  $\alpha_{1H}$  and  $\alpha_{1I}$ . An overview and comparison is given in Table 1. The complete amino acid sequences of the individual channels of one subfamily are more than 80% identical. Among the HVA channels the L-type and neuronal types share about 50% sequence homology, whereas HVA and LVA channels are less than 30% identical (Figure 2). The diversity of VDCC is not only due to the existence of different calcium channel genes and various auxiliary subunits, but also to a great number of splice variants of the  $\alpha_1$  subunit. Usually they share more than 95% sequence homology. So far, four  $\beta$  subunits ( $\beta_1 - \beta_4$ ) have been identified (for review see ref. <sup>21, 22</sup>), which each give rise to alternatively spliced products. All  $\beta$  subunits differ significantly in their N- and C-termini, but share a common central core consisting of two domains, with the second domain carrying the interaction site with the calcium channel  $\alpha_2$  subunit (BID,  $\beta$  interaction domain). Four different  $\alpha_2\delta$  subunits ( $\alpha_2\delta1 - \alpha_2\delta4$ ) have been discovered. But, although the skeletal muscle  $\alpha_2\delta1$  subunit was one of the first subunits to be cloned,  $\alpha_2\delta2$  through  $\alpha_2\delta4$  were discovered more than 20 years later. A similar pattern was found for the  $\gamma$  subunits. The first was identified in 1990<sup>48, 49</sup>. For a long time, it was considered unique to skeletal muscles. Later, additional four  $\gamma$  subunits ( $\gamma$ -2 through  $\gamma$ -5) were identified in brain, liver, heart, kidney, lung and testes<sup>46</sup>.

Table 1. Classification and nomenclature of VDCCs

family	current	former name	new name	distribution	blockers
HVA channels	L-type	$\alpha_{1S}$	$Ca_v1.1$	skeletal	DHPs (e.g. Nifedipine), PAAs (e.g. Verapamil), BTZs (e.g. Diltiazem)
		$\alpha_{1C}$	$Ca_v1.2$	heart smooth muscle <sup>1</sup>	
		$\alpha_{1D}$	$Ca_v1.3$	neuroendocrine <sup>2</sup>	
		$\alpha_{1F}$	$Ca_v1.4$	retina	
	P/Q-type	$\alpha_{1A}$	$Ca_v2.1$	neuronal	$\omega$ -Agatoxin IVA
	N-type	$\alpha_{1B}$	$Ca_v2.2$	neuronal	$\omega$ -Conotoxin GVIA
R-type	$\alpha_{1E}$	$Ca_v2.3$	neuronal	SNX 482	
LVA channels	T-type	$\alpha_{1G}$	$Ca_v3.1$	neuronal, cardiac	Mibefradil <sup>3</sup> , Kurtoxin <sup>3</sup> , Ni <sup>2+</sup>
		$\alpha_{1H}$	$Ca_v3.2$	neuronal, cardiac	Mibefradil <sup>3</sup> , Kurtoxin <sup>3</sup> , Ni <sup>2+</sup>
		$\alpha_{1I}$	$Ca_v3.3$	neuronal	Mibefradil <sup>3</sup> , Kurtoxin <sup>3</sup> , Ni <sup>2+</sup>

<sup>1</sup> also expressed in endocrine and neuronal cells, <sup>2</sup> also described in cardiac pacemaker cells and cochlear hair cells, <sup>3</sup> not highly specific

Pharmacology of native and expressed channels

There has been great interest in developing specific drugs or toxins as probes to study the structure and function of the multiple calcium channel subtypes. Verapamil (Figure 1) was the first calcium antagonist identified and named by Fleckenstein in the 1960's. It belongs to the phenylalkylamines, which act use-dependently as intracellular pore blockers. In contrast to PAAs, benzothiazepines such as Diltiazem (Figure 1) interact with extracellular sites of the calcium channel  $\alpha_1$  subunit. About 20 years after the invention of Verapamil, Fleckenstein also described nifedipine (Figure 1), the first representative of the Dihydropyridines. The binding sites of PAAs, BTZs and DHPs are formed by amino acid residues in the (fifth and) sixth transmembrane segments of domains III and IV of the  $\alpha_1$  subunit. But in contrast to PAAs and BTZs, it has been proposed that DHPs act allosterically to shift the channel toward the open or closed state instead of blocking the ion-conducting pore. Therefore, DHPs can be channel activators e.g. BayK 8644 or inhibitors (Figure 1). These drugs are effective in patients with hypertension, angina pectoris, and cardiac arrhythmias and may be beneficial in patients with left ventricular diastolic dysfunction, Raynaud's phenomenon, migraine, oesophageal spasm, and bipolar disorders<sup>74</sup>. The clinical relevant calcium channel antagonists are 100-fold selective for L-type channels. Dihydropyridines are the most selective and potent blockers of L-type calcium channels in the vascular system<sup>75</sup>. Interestingly, myocardial and vascular smooth muscle cells express the same L-type channels. One explanation for the higher selectivity of DHPs for the vascular smooth muscle is that these drugs preferentially block inactivated channels. The lower resting potential of the smooth muscle shifts the channels into the inactivated state. Later, it could be shown that the difference was also persistent, when only "smooth muscle" or "cardiac"  $\alpha_1$  subunits were expressed in cell lines<sup>76</sup>. Molecular analysis revealed that the alternatively spliced exon 8, which codes for the IS6 segment, is differentially expressed in cardiac and smooth muscle. By means of mutational analysis it could be proven that these IS6 segments were involved in the different DHP sensitivity of the cardiac and the smooth muscle L-type channel<sup>76-79</sup>. Alternative splicing is also involved in the voltage dependence of DHP action<sup>80,81</sup>.

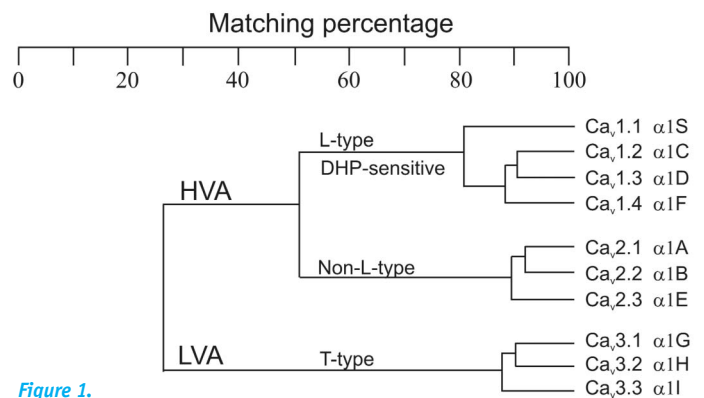


Figure 1. Phylogenetic tree of VDCC

N-type ( $Ca_v2.2$ ) calcium channels were first cloned in the 90's<sup>58,82,83</sup>. They are highly concentrated in presynaptic nerve terminals and dendrites and mediate multiple cellular functions in neurons, including neurotransmitter release<sup>84-86</sup>. A variety of neurotoxins has been described to block neuronal calcium channels. The best-known example of a N-type blocker is  $\omega$ -Conotoxin GVIA (Figure 1) from *Conus geographus*, a member of a large family of peptide toxins derived from venomous cone snails<sup>87</sup>. The pharmacological method was the first to distinguish N-type calcium current in different tissue, because the voltage dependence and kinetics of N-type calcium currents vary considerably among neurons<sup>83</sup>. The blockade of calcium currents by  $\omega$ -Conotoxin GVIA showed a potent antinociceptive effect<sup>88,89</sup>. Other peptide toxins identified further neuronal channels. P-type calcium currents which were first recorded in Purkinje neurons are highly sensitive to the spider toxin  $\omega$ -Agatoxin IVA (Figure 1)<sup>90-92</sup>. The same toxin blocks Q-type currents in cerebellar granular neurons with lower affinity<sup>93</sup>.  $\omega$ -Conotoxin MVIIC inhibits reversibly N-type currents and irreversibly both P- and Q-type calcium currents<sup>94</sup>.

## Voltage-Dependent Calcium Channels

R-type channels, which were first identified in cerebellar granula cells<sup>95, 96</sup> are heterogeneous, consisting of elements that result from the expression of  $\alpha_{1E}$  as well as other non- $\alpha_{1E}$ -dependent components<sup>97</sup>. Originally, they were defined as channels “resistant” to blockers for L-, N- and P/Q-type channels. For a long time, no specific toxin or drug was available to target the “residual” calcium current. Recently, SNX 482 (Figure 1), a toxin from the venom of the tarantula *Hysterocrates gigas* has been described that blocks the R-type calcium channel in heterologous expression systems<sup>98</sup>, but is not entirely effective on R-type currents in every native neuronal tissue. The T-type calcium currents are insensitive to both the L-type calcium channel blockers and the spider and snail toxins. There are no widely useful agents that inhibit LVA channels<sup>24</sup>. Among the inorganic cations, Ni<sup>2+</sup> and Cd<sup>2+</sup> represent the most specific blockers for LVA channels. The only organic blocker with a higher selectivity for T-type calcium channels is mibefradil<sup>99, 100</sup>. The drug inhibits also HVA channels, but with a somewhat lower affinity<sup>54, 101</sup>. It was withdrawn from the market in 1998 due to the potential for drug interactions. Recently, another organic T-type blocker, the alpha-scorpion toxin kurtoxin, was described which blocks the Ca<sub>v</sub>3.1 channel with an IC<sub>50</sub> value of 15 nM by interfering with the activation gate of the channel<sup>102</sup>. Some antiepileptics may also develop their effects by blockade of T-type channels (for review see ref. <sup>20, 21</sup>). Thus, ethosuximide can block thalamic T-type currents and reduce oscillations<sup>103</sup>. Expressed channels show a moderate sensitivity versus valproate and phenytoin. Whether gabapentin, which is useful in the treatment of partial seizures, exerts its effect via T-type channels is not fully understood. It has been shown that the drug binds to the  $\alpha_2\delta$  subunit with high affinity<sup>104</sup>. Development of more specific T-type blockers would be very useful for therapy. In addition, high affinity inhibitors would help to analyse the function of HVA channels in native tissues, where HVA currents usually mask their activity.

### Calcium channelopathies

Channelopathies are diseases caused by (inherited) mutations in genes that encode ion channel subunits or regulatory proteins<sup>105</sup>. Due to the different nature of the ion channels the channelopathies range from inherited cardiac arrhythmias, to muscle disorders, to forms of diabetes. They are listed in **OMIM**, the **Mendelian Inheritance in Man** project. This is a database provided by the Johns Hopkins University that catalogues all the known diseases with a genetic component, and -when possible- links them to the relevant genes in the human genome and provides references for further research and tools for genomic analysis of a catalogued gene. For a long time, calcium channelopathies were only described in channels with a restricted expression, for example Ca<sub>v</sub>1.1 in skeletal muscle or Ca<sub>v</sub>1.4 in the retina (for review see ref. <sup>106, 107</sup> and Table 2). The first calcium channelopathy described in humans was the hypokalemic periodic paralysis type 1, linked to the skeletal muscle calcium channel<sup>108, 109</sup>. Missense mutations within the S4 voltage sensor segments result in a reduced calcium current density. Other mutations in the CACNA1S gene rather led to a gain of function like in malignant hyperthermia. Genetic linkage analysis of congenital stationary night blindness type 2 (CSNB2) identified CACNA1F coding for Ca<sub>v</sub>1.4, a novel calcium channel expressed exclusively in the retina<sup>59, 60</sup>. To date, over sixty CSNB2 mutations have been described in CACNA1F. Recently, another retinal disease, X-linked cone-rod dystrophy type 3, was also allocated to CACNA1F channelopathies<sup>110</sup>.

Among the neuronal HVA calcium channels, only mutations in CACNA1A coding for Ca<sub>v</sub>2.1 have been identified to date, leading to specific forms of migraine, seizure and ataxia syndromes (for review see ref. <sup>111, 112</sup>). Similar neurological dysfunctions are shown by spontaneous autosomal recessive mouse mutants (*tottering*, *leaner*, *rolling and rocker*), whereas spontaneous mutations in mouse Ca<sub>v</sub>2.2 or Ca<sub>v</sub>2.3  $\alpha_1$  subunit have not been described<sup>111, 113</sup>.

**Table 2. Human inherited calcium channelopathies<sup>1</sup>**

family	channel	gene	disease	OMIM
HVA channels	Ca <sub>v</sub> 1.1	CACNA1S	Hypokalemic periodic paralysis type 1 Malignant Hyperthermia type 5	HypoPP1 MHS5 170400 601887
	Ca <sub>v</sub> 1.2	CACNA1C	Timothy syndrome	TS 601005
	Ca <sub>v</sub> 1.3	CACNA1D	-	
	Ca <sub>v</sub> 1.4	CACNA1F	Congenital stationary night blindness type 2 X-linked cone-rod dystrophy type 3	CSNB2 CORDX3 300071 300476
	Ca <sub>v</sub> 2.1	CACNA1A	Familial hemiplegic migraine type 1 Episodic ataxia type 2 Spinocerebellar ataxia type 6	FHM1 EA-2 SCA6 141500 108500 183086
	Ca <sub>v</sub> 2.2	CACNA1B	-	
	Ca <sub>v</sub> 2.3	CACNA1E	-	
LVA channels	Ca <sub>v</sub> 3.1	CACNA1G	-	
	Ca <sub>v</sub> 3.2	CACNA1H	Childhood absence epilepsy Autism spectrum disorders	CAE ASD
	Ca <sub>v</sub> 3.3	CACNA1I	-	

<sup>1</sup> for detailed review see Bidaud et al. 2006, Striessnig et al. 2004<sup>106, 107</sup>

Of the three T-type calcium channel genes, only mutations in *CACNA1H*, which codes for the  $Ca_v3.2$  subunit, have been identified. They manifest themselves as childhood absence epilepsies and autism spectrum disorders (ASD). One third of the ASD patients also suffer from epilepsy<sup>106</sup>.

For channels with ubiquitous expression, like  $Ca_v1.2$  severe phenotypes were expected which may be incompatible with life. In line with this  $Ca_v1.2$  knockout mice die in utero before day 14.5 p.c.<sup>114</sup>. Therefore, such a defect may also lead to intrauterine death in humans. Anyhow, mutations in *CACNA1C* gene, which code for the  $Ca_v1.2$  subunit, have been recently identified. The resulting disease was named Timothy syndrome. It represents a rare childhood multi-organ disorder with severe cardiac defects and sudden death. In addition, syndactyly, immune deficiency, intermitted hypoglycaemia, cognitive abnormalities and autism occurred in patients<sup>115, 116</sup>. It is linked to *de novo* missense mutation in exon 8A of the  $Ca_v1.2$  subunit leading to the formation of a consensus sequence for CaMKII phosphorylation which results in a failure of channel inactivation<sup>117, 118</sup>. Therefore this “gain of function mutation” leads to a delay in action potential repolarisation and thus to long QT syndrome. The calcium channelopathies are summarized in Table 2. The increasing understanding of the pathogenic channel mutations should lead to improved treatments of such hereditary diseases in humans.

#### New insight from transgenic mice and conditional mutagenesis

The introduction of knockout mice and conditional mutagenesis has given new and interesting insight into the expression and function of calcium channels<sup>5, 119</sup>. Especially for the L-type calcium channels genetically modified mice turned out to be very useful, because the isoforms lack selective modulators and thus it is impossible to determine their relative contribution in different tissues. In homozygous  $Ca_v1.3 \alpha_1$  knockout mice, a complete loss of  $Ca_v1.3$  function led to sinoatrial and AV-node dysfunction and a complete hearing loss<sup>120, 121</sup>. A mouse model in which the DHP binding site was eliminated in  $Ca_v1.2$  allowed isolating the function of  $Ca_v1.3$  in different tissues. These studies ruled out a direct role for  $Ca_v1.3$  in insulin secretion, cardiac inotropy, and arterial smooth muscle contractility. Instead it was shown that  $Ca_v1.3$  plays a role in depression<sup>122</sup>. In heart the most abundant HVA calcium channel is  $Ca_v1.2$ . It contributes essential to the plateau of the cardiac action potential. Accordingly,  $Ca_v1.2$  knockout mice die in utero before day 14.5 p.c. due to cardiac dysfunction<sup>114</sup>. However, contraction of transgenic and wild type embryonic hearts was similar till day 12.5 post coitum. In agreement with this, a novel  $Ca_v1.3$  splice variant with a lower affinity to DHPs could be cloned from transgenic embryonic hearts<sup>123</sup>. In knockout hearts, this subunit was up-regulated, but could not compensated for the loss of  $Ca_v1.2$  after day 14.5<sup>124</sup>. Further support for the role of  $Ca_v1.2$  in cardiac development comes from a study by Rottbauer et al.<sup>125</sup>. Genetic mutations in the zebrafish  $Ca_v1.2$  calcium channel subunit led to a lethal abnormal heart growth during development.

To study the roles of  $Ca_v1.2$  in vivo, several mouse lines were created with time- or tissue-dependent conditional inactivation of the  $Ca_v1.2$  gene<sup>3, 126-130</sup>. With the help of these mice, it could be shown that  $Ca_v1.2$  is essential for blood pressure regulation, bladder and intestine contraction, and even spatial learning. Interestingly and in agreement with the  $Ca_v1.3$  knockout studies, it was found that the ablation of  $Ca_v1.2$  in pancreatic  $\beta$ -cells led to attenuation of the first phase of insulin secretion. This phenomenon is one of the key symptoms in type-2 diabetes mellitus. Therefore, it was not surprising that  $\beta Ca_v1.2^{-/-}$  mice suffer from a slight hyperglycaemia under basal and fasted conditions and an impaired glucose tolerance after intraperitoneal glucose challenge. In pancreatic  $\beta$ -cells,  $Ca_v1.2$  is the only L-type calcium channel, whereas in glucagon secreting  $\alpha$ -cells both,  $Ca_v1.2$  and  $Ca_v1.3$ , were identified with the help of knock out mice and the calcium channel blocker isradipine (Figure 1)<sup>128</sup>. This may explain why  $Ca_v1.3$  could be cloned from pancreatic islet cells and offer the possibility that  $Ca_v1.2$  as well as  $Ca_v1.3$  are involved in glucagon secretion. Another calcium channel, putatively involved in glucagon secretion is  $Ca_v2.2$ <sup>131</sup>.  $Ca_v2.2$  channel-deficient mice showed an increased glucose tolerance and a reduced weight gain without any change in insulin sensitivity under high-fat diet<sup>132</sup>. Others didn't identify  $Ca_v2.2$  in pancreatic  $\alpha$ -cells<sup>128</sup>. In contrast, high levels of  $Ca_v2.3$  current were found.  $Ca_v2.3$  deficient mice showed markedly reduced glucose tolerance and impaired insulin secretion, due to a disturbance in the second phase of insulin secretion<sup>133-135</sup>. A role of  $Ca_v2.3$  for glucagon secretion has yet to be established.

All in all,  $Ca_v2.2^{-/-}$  and  $Ca_v2.3^{-/-}$  mice are healthy, fertile and do not show any overt neurological phenotype. It has been suggested that other types of calcium channels must be able to compensate for the loss. Nevertheless, functional analysis revealed some dysfunctions in pain and drug response, hyperactivity and increased anxiety, as well as impaired learning and memory<sup>89, 97, 111, 113, 134, 136, 137</sup>.

$Ca_v2.1$  knockout mice die during the first 4 weeks after birth, suffering from ataxia, dystonia, absence epilepsy and progressive cerebellar degeneration<sub>v</sub>. These mice provided the direct evidence that the gene encodes the P/Q-type calcium current.

Targeted disruption of the  $Ca_v3.1$  gene proved the critical role of  $\alpha_{1G}$  in the generation of absence seizures in the thalamocortical network and in wakefulness and arousal<sup>133, 140</sup>. In the heart,  $Ca_v3.1^{-/-}$  mice lacked the T-type calcium current in both sinoatrial pacemaker and AV node cells and displayed slowed pacemaker activity and AV conduction<sup>141</sup>. Total deletion of  $Ca_v3.2$  led to abnormal coronary function<sup>142</sup>.

Deletion of the different calcium channel auxiliary subunits resulted in a broad spectrum of defects. The targeted disruption of  $Ca_v\beta_1$ , the main  $\beta$  subunit in skeletal muscle, led to decreased L-type currents, skeletal muscle dysfunction and death at birth due to an asphyxia<sup>143</sup>. Even worse, the disruption of the main cardiac  $\beta_2$  subunit, caused embryonic death around day 11 post coitum, due to an abnormal cardiac development<sup>144</sup>. Deletion of  $Ca_v\beta_3$  or  $\gamma_1$  led to either a decreased or increased L- (and N-type) calcium current, respectively, with no further identifiable pathology<sup>50, 145, 146</sup>.

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## Voltage-Dependent Calcium Channels

### Perspectives

Voltage-dependent calcium channels are key regulators in many cellular processes like cell contraction, transmitter release, gene transcription and hormone secretion. Therefore, not surprising calcium channels are involved in diseases like hypertension, cardiac arrhythmias, migraine or depression. Mutations in the various calcium channel genes are lethal or lead to severe diseases, the channelopathies. Thus, the development of drugs and toxins that selectively target the different calcium channels appear to be particularly important but aggravated by the many splice variants of the various calcium channel subunits that have been identified. Their different combinations result in calcium currents with different biophysical properties. Knockout and transgenic mice have given new insight into the distribution and function of the voltage-dependent calcium channels. These mice will be useful models to study the effects of the different calcium channel blockers and activators and will help to develop new and more specific drugs.

*Abbreviations: ASD, autism spectrum disorders; AV, atrioventricular; DHP, dihydropyridine; HVA, high voltage activated; LVA, low voltage activated; PAA; phenylalkylamine; BTZ, benzothiazepine; VDCC, voltage-dependent calcium channel.*

## References

- 001 Tanabe, T., Beam, K. G., Adams, B. A., et al. (1990) Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature* 346, 567-569
- 002 Dolmetsch, R. E., Pajvani, U., Fife, K., et al. (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* 294, 333-339
- 003 Moosmang, S., Haider, N., Klugbauer, N., et al. (2005) Role of hippocampal Cav1.2 Ca<sup>2+</sup> channels in NMDA receptor-independent synaptic plasticity and spatial memory. *J Neurosci* 25, 9883-9892
- 004 Artalejo, C. R., Adams, M. E. and Fox, A. P. (1994) Three types of Ca<sup>2+</sup> channel trigger secretion with different efficacies in chromaffin cells. *Nature* 367, 72-76
- 005 Moosmang, S., Kleppisch, T., Wegener, J., et al. (2007) Analysis of calcium channels by conditional mutagenesis. *Handb Exp Pharmacol*, 469-490
- 006 Fatt, P. and Katz, B. (1953) The effect of inhibitory nerve impulses on a crustacean muscle fibre. *J Physiol* 121, 374-389
- 007 Takahashi, M., Seagar, M. J., Jones, J. F., et al. (1987) Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proc Natl Acad Sci U S A* 84, 5478-5482
- 008 Tanabe, T., Takeshima, H., Mikami, A., et al. (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 328, 313-318
- 009 Borsotto, M., Barhanin, J., Fosset, M., et al. (1985) The 1,4-dihydropyridine receptor associated with the skeletal muscle voltage-dependent Ca<sup>2+</sup> channel. Purification and subunit composition. *J Biol Chem* 260, 14255-14263
- 010 Flockerzi, V., Oeken, H. J. and Hofmann, F. (1986) Purification of a functional receptor for calcium-channel blockers from rabbit skeletal-muscle microsomes. *Eur J Biochem* 161, 217-224
- 011 Curtis, B. M. and Catterall, W. A. (1984) Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry* 23, 2113-2118
- 012 Biel, M., Ruth, P., Bosse, E., et al. (1990) Primary structure and functional expression of a high voltage activated calcium channel from rabbit lung. *FEBS Lett* 269, 409-412.
- 013 Mikami, A., Imoto, K., Tanabe, T., et al. (1989) Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature* 340, 230-233
- 014 Nowicky, M. C., Fox, A. P. and Tsien, R. W. (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature* 316, 440-443
- 015 Fox, A. P., Nowicky, M. C. and Tsien, R. W. (1987) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J Physiol* 394, 149-172
- 016 Bean, B. P. (1989) Classes of calcium channels in vertebrate cells. *Annu Rev Physiol* 51, 367-384
- 017 Hess, P. (1990) Calcium channels in vertebrate cells. *Annu Rev Neurosci* 13, 337-356
- 018 McCleskey, E. W., Fox, A. P., Feldman, D. H., et al. (1987) Omega-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc Natl Acad Sci U S A* 84, 4327-4331
- 019 Tsien, R. W., Lipscombe, D., Madison, D. V., et al. (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci* 11, 431-438
- 020 Lacinova, L., Klugbauer, N. and Hofmann, F. (2000) Low voltage activated calcium channels: from genes to function. *Gen Physiol Biophys* 19, 121-136
- 021 Lacinova, L. (2005) Voltage-dependent calcium channels. *Gen Physiol Biophys* 24 Suppl 1, 1-78
- 022 Hofmann, F., Lacinova, L. and Klugbauer, N. (1999) Voltage-dependent calcium channels: from structure to function. *Rev. Physiol. Biochem. Pharmacol.* 139, 33-87
- 023 Catterall, W. A. (2000) Structure and regulation of voltage-gated Ca<sup>2+</sup> channels. *Annu Rev Cell Dev Biol* 16, 521-555
- 024 Perez-Reyes, E. (2003) Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev* 83, 117-161
- 025 Dolphin, A. C. (2006) A short history of voltage-gated calcium channels. *Br J Pharmacol* 147 Suppl 1, S56-62
- 026 Guy, H. R. and Conti, F. (1990) Pursuing the structure and function of voltage-gated channels. *Trends Neurosci* 13, 201-206
- 027 Heinemann, S. H., Terlau, H., Stuhmer, W., et al. (1992) Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature* 356, 441-443
- 028 Jiang, Y., Ruta, V., Chen, J., et al. (2003) The principle of gating charge movement in a voltage-dependent K<sup>+</sup> channel. *Nature* 423, 42-48
- 029 Guy, H. R. and Seetharamulu, P. (1986) Molecular model of the action potential sodium channel. *Proc Natl Acad Sci U S A* 83, 508-512
- 030 Pragnell, M., De Waard, M., Mori, Y., et al. (1994) Calcium channel beta-subunit binds to a conserved motif in the I-II cytoplasmic linker of the alpha 1-subunit. *Nature* 368, 67-70
- 031 Ruth, P., Rohrkasten, A., Biel, M., et al. (1989) Primary structure of the beta-subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science* 245, 1115-1118
- 032 Welling, A., Bosse, E., Cavalie, A., et al. (1993) Stable co-expression of calcium channel alpha 1, beta and alpha 2/delta subunits in a somatic cell line. *J Physiol* 471, 749-765
- 033 Singer, D., Biel, M., Lotan, I., et al. (1991) The roles of the subunits in the function of the calcium channel. *Science* 253, 1553-1557
- 034 Neely, A., Wei, X., Olcese, R., et al. (1993) Potentiation by the beta subunit of the ratio of the ionic current to the charge movement in the cardiac calcium channel. *Science* 262, 575-578
- 035 Hullin, R., Singer-Lahat, D., Freichel, M., et al. (1992) Calcium channel beta-subunit heterogeneity: functional expression of cloned cDNA from heart, aorta and brain. *Embo J* 11, 885-890
- 036 Josephson, I. R. and Varadi, G. (1996) The beta subunit increases Ca<sup>2+</sup> currents and gating charge movements of human cardiac L-type Ca<sup>2+</sup> channels. *Biophys J* 70, 1285-1293
- 037 Kamp, T. J., Perez-Garcia, M. T. and Marban, E. (1996) Enhancement of ionic current and charge movement by coexpression of calcium channel beta 1A subunit with alpha 1C subunit in a human embryonic kidney cell line. *J Physiol* 492 ( Pt 1), 89-96
- 038 Dolphin, A. C. (2003) Beta subunits of voltage-gated calcium channels. *J Bioenerg Biomembr* 35, 599-620
- 039 Wei, X. Y., Perez-Reyes, E., Lacerda, A. E., et al. (1991) Heterologous regulation of the cardiac Ca<sup>2+</sup> channel alpha 1 subunit by skeletal muscle beta and gamma subunits. Implications for the structure of cardiac L-type Ca<sup>2+</sup> channels. *J Biol Chem* 266, 21943-21947
- 040 Lacerda, A. E., Kim, H. S., Ruth, P., et al. (1991) Normalization of current kinetics by interaction between the alpha 1 and beta subunits of the skeletal muscle dihydropyridine-sensitive Ca<sup>2+</sup> channel. *Nature* 352, 527-530
- 041 Ellis, S. B., Williams, M. E., Ways, N. R., et al. (1988) Sequence and expression of mRNAs encoding the alpha 1 and alpha 2 subunits of a DHP-sensitive calcium channel. *Science* 241, 1661-1664
- 042 Gurnett, C. A., De Waard, M. and Campbell, K. P. (1996) Dual function of the voltage-dependent Ca<sup>2+</sup> channel alpha 2 delta subunit in current stimulation and subunit interaction. *Neuron* 16, 431-440
- 043 Klugbauer, N., Lacinova, L., Marais, E., et al. (1999) Molecular diversity of the calcium channel alpha2delta subunit. *J Neurosci* 19, 684-691
- 044 Angelotti, T. and Hofmann, F. (1996) Tissue-specific expression of splice variants of the mouse voltage-gated calcium channel alpha2/delta subunit. *FEBS Lett* 397, 331-337
- 045 Bangalore, R., Mehrke, G., Gingrich, K., et al. (1996) Influence of L-type Ca channel alpha 2/delta-subunit on ionic and gating current in transiently transfected HEK 293 cells. *Am J Physiol Heart Circ Physiol* 270, H1521-1528
- 046 Klugbauer, N., Dai, S., Specht, V., et al. (2000) A family of gamma-like calcium channel subunits. *FEBS Lett* 470, 189-197
- 047 Letts, V. A., Felix, R., Biddlecome, G. H., et al. (1998) The mouse stargazer gene encodes a neuronal Ca<sup>2+</sup>-channel gamma subunit. *Nat Genet* 19, 340-347
- 048 Bosse, E., Regulla, S., Biel, M., et al. (1990) The cDNA and deduced amino acid sequence of the gamma subunit of the L-type calcium channel from rabbit skeletal muscle. *FEBS Lett* 267, 153-156
- 049 Jay, S. D., Ellis, S. B., McCue, A. F., et al. (1990) Primary structure of the gamma subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science* 248, 490-492
- 050 Andronache, Z., Ursu, D., Lehnert, S., et al. (2007) The auxiliary subunit gamma 1 of the skeletal muscle L-type Ca<sup>2+</sup> channel is an endogenous Ca<sup>2+</sup> antagonist. *Proc Natl Acad Sci U S A* 104, 17885-17890
- 051 Bosse, E., Bottlender, R., Kleppisch, T., et al. (1992) Stable and functional expression of the calcium channel alpha 1 subunit from smooth muscle in somatic cell lines. *Embo J* 11, 2033-2038
- 052 Welling, A., Bosse, E., Ruth, P., et al. (1992) Expression and regulation of cardiac and smooth muscle calcium channels. *Jpn J Pharmacol* 58 Suppl 2, 258P-262P

## References

- 053 Perez-Reyes, E., Kim, H. S., Lacerda, A. E., et al. (1989) Induction of calcium currents by the expression of the alpha 1-subunit of the dihydropyridine receptor from skeletal muscle. *Nature* 340, 233-236
- 054 Welling, A., Lacinova, L., Donatin, K., et al. (1995) Expression of the L-type calcium channel with two different beta subunits and its modulation by Ro 40-5967. *Pflugers Arch* 429, 400-411
- 055 Lacinova, L., Welling, A., Bosse, E., et al. (1995) Interaction of Ro 40-5967 and verapamil with the stably expressed alpha 1-subunit of the cardiac L-type calcium channel. *J Pharmacol Exp Ther* 274, 54-63.
- 056 Zamponi, G. W., Soong, T. W., Bourinet, E., et al. (1996) Beta subunit co-expression and the alpha1 subunit domain I-II linker affect piperidine block of neuronal calcium channels. *J Neurosci* 16, 2430-2443
- 057 Seino, S., Chen, L., Seino, M., et al. (1992) Cloning of the alpha 1 subunit of a voltage-dependent calcium channel expressed in pancreatic beta cells. *Proc Natl Acad Sci U S A* 89, 584-588
- 058 Williams, M. E., Feldman, D. H., McCue, A. F., et al. (1992) Structure and functional expression of alpha 1, alpha 2, and beta subunits of a novel human neuronal calcium channel subtype. *Neuron* 8, 71-84
- 059 Bech-Hansen, N. T., Naylor, M. J., Maybaum, T. A., et al. (1998) Loss-of-function mutations in a calcium-channel alpha1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat Genet* 19, 264-267
- 060 Strom, T. M., Nyakatura, G., Apfelstedt-Sylla, E., et al. (1998) An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nat Genet* 19, 260-263
- 061 Starr, T. V., Prystay, W. and Snutch, T. P. (1991) Primary structure of a calcium channel that is highly expressed in the rat cerebellum. *Proc Natl Acad Sci U S A* 88, 5621-5625
- 062 Mori, Y., Friedrich, T., Kim, M. S., et al. (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature* 350, 398-402
- 063 Horne, W. A., Ellinor, P. T., Inman, I., et al. (1993) Molecular diversity of Ca<sup>2+</sup> channel alpha 1 subunits from the marine ray *Discopyge ommata*. *Proc Natl Acad Sci U S A* 90, 3787-3791
- 064 Williams, M. E., Brust, P. F., Feldman, D. H., et al. (1992) Structure and functional expression of an omega-conotoxin-sensitive human N-type calcium channel. *Science* 257, 389-395
- 065 Soong, T. W., Stea, A., Hodson, C. D., et al. (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* 260, 1133-1136
- 066 Williams, M. E., Marubio, L. M., Deal, C. R., et al. (1994) Structure and functional characterization of neuronal alpha 1E calcium channel subtypes. *J Biol Chem* 269, 22347-22357
- 067 Perez-Reyes, E., Cribbs, L. L., Daud, A., et al. (1998) Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. *Nature* 391, 896-900
- 068 Cribbs, L. L., Martin, B. L., Schroder, E. A., et al. (2001) Identification of the t-type calcium channel Ca<sub>v</sub>3.1d in developing mouse heart. *Circ Res* 88, 403-407
- 069 Klugbauer, N., Marais, E., Lacinova, L., et al. (1999) A T-type calcium channel from mouse brain. *Pflugers Arch* 437, 710-715
- 070 Lee, J. H., Daud, A. N., Cribbs, L. L., et al. (1999) Cloning and expression of a novel member of the low voltage-activated T-type calcium channel family. *J Neurosci* 19, 1912-1921
- 071 Birnbaumer, L., Campbell, K. P., Catterall, W. A., et al. (1994) The naming of voltage-gated calcium channels. *Neuron* 13, 505-506
- 072 Ertel, E. A., Campbell, K. P., Harpold, M. M., et al. (2000) Nomenclature of voltage-gated calcium channels [letter]. *Neuron* 25, 533-535
- 073 Catterall, W. A., Perez-Reyes, E., Snutch, T. P., et al. (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev* 57, 411-425
- 074 Abernethy, D. R. and Schwartz, J. B. (1999) Calcium-antagonist drugs. *N Engl J Med* 341, 1447-1457
- 075 Fleckenstein, A. (1983) History of calcium antagonists. *Circ Res* 52, I3-16
- 076 Welling, A., Kwan, Y. W., Bosse, E., et al. (1993) Subunit-dependent modulation of recombinant L-type calcium channels. Molecular basis for dihydropyridine tissue selectivity. *Circ Res* 73, 974-980
- 077 Welling, A., Ludwig, A., Zimmer, S., et al. (1997) Alternatively spliced IS6 segments of the alpha 1C gene determine the tissue-specific dihydropyridine sensitivity of cardiac and vascular smooth muscle L-type Ca<sup>2+</sup> channels. *Circ Res* 81, 526-532
- 078 Morel, N., Buryi, V., Feron, O., et al. (1998) The action of calcium channel blockers on recombinant L-type calcium channel alpha1-subunits. *Br J Pharmacol* 125, 1005-1012
- 079 Hu, H. and Marban, E. (1998) Isoform-specific inhibition of L-type calcium channels by dihydropyridines is independent of isoform-specific gating properties. *Mol Pharmacol* 53, 902-907
- 080 Soldatov, N. M., Bouron, A. and Reuter, H. (1995) Different voltage-dependent inhibition by dihydropyridines of human Ca<sup>2+</sup> channel splice variants. *J Biol Chem* 270, 10540-10543
- 081 Zuhlke, R. D., Bouron, A., Soldatov, N. M., et al. (1998) Ca<sup>2+</sup> channel sensitivity towards the blocker isradipine is affected by alternative splicing of the human alpha1C subunit gene. *FEBS Lett* 427, 220-224
- 082 McEnery, M. W., Snowman, A. M., Sharp, A. H., et al. (1991) Purified omega-conotoxin GVIA receptor of rat brain resembles a dihydropyridine-sensitive L-type calcium channel. *Proc Natl Acad Sci U S A* 88, 11095-11099
- 083 Fujita, Y., Mynlieff, M., Dirksen, R. T., et al. (1993) Primary structure and functional expression of the omega-conotoxin-sensitive N-type calcium channel from rabbit brain. *Neuron* 10, 585-598
- 084 Westenbroek, R. E., Hell, J. W., Warner, C., et al. (1992) Biochemical properties and subcellular distribution of an N-type calcium channel alpha 1 subunit. *Neuron* 9, 1099-1115
- 085 Westenbroek, R. E., Hoskins, L. and Catterall, W. A. (1998) Localization of Ca<sup>2+</sup> channel subtypes on rat spinal motor neurons, interneurons, and nerve terminals. *J Neurosci* 18, 6319-6330
- 086 Wheeler, D. B., Randall, A. and Tsien, R. W. (1994) Roles of N-type and Q-type Ca<sup>2+</sup> channels in supporting hippocampal synaptic transmission. *Science* 264, 107-111
- 087 Olivera, B. M., McIntosh, J. M., Cruz, L. J., et al. (1984) Purification and sequence of a presynaptic peptide toxin from *Conus geographus* venom. *Biochemistry* 23, 5087-5090
- 088 Malmberg, A. B. and Yaksh, T. L. (1995) Effect of continuous intrathecal infusion of omega-conopeptides, N-type calcium-channel blockers, on behavior and antinociception in the formalin and hot-plate tests in rats. *Pain* 60, 83-90
- 089 Kim, C., Jun, K., Lee, T., et al. (2001) Altered nociceptive response in mice deficient in the alpha(1B) subunit of the voltage-dependent calcium channel. *Mol Cell Neurosci* 18, 235-245
- 090 Llinas, R. R., Sugimori, M. and Cherksey, B. (1989) Voltage-dependent calcium conductances in mammalian neurons. The P channel. *Ann N Y Acad Sci* 560, 103-111
- 091 Mintz, I. M., Adams, M. E. and Bean, B. P. (1992) P-type calcium channels in rat central and peripheral neurons. *Neuron* 9, 85-95
- 092 Mintz, I. M., Venema, V. J., Swiderek, K. M., et al. (1992) P-type calcium channels blocked by the spider toxin omega-Aga-IVA. *Nature* 355, 827-829
- 093 Randall, A. and Tsien, R. W. (1995) Pharmacological dissection of multiple types of Ca<sup>2+</sup> channel currents in rat cerebellar granule neurons. *J Neurosci* 15, 2995-3012
- 094 Liu, H., De Waard, M., Scott, V. E., et al. (1996) Identification of three subunits of the high affinity omega-conotoxin MVIIC-sensitive Ca<sup>2+</sup> channel. *J Biol Chem* 271, 13804-13810
- 095 Zhang, J. F., Randall, A. D., Ellinor, P. T., et al. (1993) Distinctive pharmacology and kinetics of cloned neuronal Ca<sup>2+</sup> channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology* 32, 1075-1088
- 096 Piedras-Renteria, E. S. and Tsien, R. W. (1998) Antisense oligonucleotides against alpha1E reduce R-type calcium currents in cerebellar granule cells. *Proc Natl Acad Sci U S A* 95, 7760-7765
- 097 Wilson, S. M., Toth, P. T., Oh, S. B., et al. (2000) The status of voltage-dependent calcium channels in alpha 1E knock-out mice. *J Neurosci* 20, 8566-8571
- 098 Newcomb, R., Szoke, B., Palma, A., et al. (1998) Selective Peptide Antagonist of the Class E Calcium Channel from the Venom of the Tarantula *Hysteroecrates gigas*. *Biochemistry* 37, 15353-15362
- 099 Mehrke, G., Zong, X. G., Flockerzi, V., et al. (1994) The Ca(++)-channel blocker Ro 40-5967 blocks differently T-type and L-type Ca<sup>++</sup> channels. *J Pharmacol Exp Ther* 271, 1483-1488.
- 100 Ertel, S. I. and Clozel, J. P. (1997) Mibefradil (Ro 40-5967): the first selective T-type Ca<sup>2+</sup> channel blocker. *Expert Opin Investig Drugs* 6, 569-582
- 101 Moosmang, S., Haider, N., Brudler, B., et al. (2006) Antihypertensive effects of the putative T-type calcium channel antagonist mibefradil are mediated by the L-type calcium channel Cav1.2. *Circ Res* 98, 105-110
- 102 Chuang, R. S., Jaffe, H., Cribbs, L., et al. (1998) Inhibition of T-type voltage-gated calcium channels by a new scorpion toxin. *Nat Neurosci* 1, 668-674

- 103 Huguenard, J. R. (1996) Low-threshold calcium currents in central nervous system neurons. *Annu Rev Physiol* 58, 329-348
- 104 Gee, N. S., Brown, J. P., Dissanayake, V. U., et al. (1996) The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J Biol Chem* 271, 5768-5776
- 105 Kass, R. S. (2005) The channelopathies: novel insights into molecular and genetic mechanisms of human disease. *J Clin Invest* 115, 1986-1989
- 106 Bidaud, I., Mezghrani, A., Swayne, L. A., et al. (2006) Voltage-gated calcium channels in genetic diseases. *Biochim Biophys Acta* 1763, 1169-1174
- 107 Striessnig, J., Hoda, J. C., Koschak, A., et al. (2004) L-type  $Ca^{2+}$  channels in  $Ca^{2+}$  channelopathies. *Biochem Biophys Res Commun* 322, 1341-1346
- 108 Jurkat-Rott, K., Lehmann-Horn, F., Elbaz, A., et al. (1994) A calcium channel mutation causing hypokalemic periodic paralysis. *Hum Mol Genet* 3, 1415-1419
- 109 Lehmann-Horn, F. and Jurkat-Rott, K. (1999) Voltage-Gated Ion Channels and Hereditary Disease. *Physiol. Rev.* 79, 1317-1372
- 110 Jalkanen, R., Mantjarvi, M., Tobias, R., et al. (2006) X linked cone-rod dystrophy, *CORDX3*, is caused by a mutation in the *CACNA1F* gene. *J Med Genet* 43, 699-704
- 111 Pietrobon, D. (2005) Function and dysfunction of synaptic calcium channels: insights from mouse models. *Curr Opin Neurobiol* 15, 257-265
- 112 Pietrobon, D. (2002) Calcium channels and channelopathies of the central nervous system. *Mol Neurobiol* 25, 31-50
- 113 Liu, L., Zwingman, T. A. and Fletcher, C. F. (2003) In vivo analysis of voltage-dependent calcium channels. *J Bioenerg Biomembr* 35, 671-685
- 114 Seisenberger, C., Specht, V., Welling, A., et al. (2000) Functional embryonic cardiomyocytes after disruption of the L-type alpha1C (*Cav1.2*) calcium channel gene in the mouse. *J Biol Chem* 275, 39193-39199
- 115 Splawski, I., Timothy, K. W., Decher, N., et al. (2005) Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci U S A* 102, 8089-8096; discussion 8086-8088
- 116 Splawski, I., Timothy, K. W., Sharpe, L. M., et al. (2004) *Cav1.2* calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 119, 19-31
- 117 Lee, T.-S., Karl, R., Moosmang, S., et al. (2006) Calmodulin Kinase II Is Involved in Voltage-dependent Facilitation of the L-type *Cav1.2* Calcium Channel: IDENTIFICATION OF THE PHOSPHORYLATION SITES 10.1074/jbc.M508661200. *J Biol Chem* 281, 25560-25567
- 118 Erxleben, C., Liao, Y., Gentile, S., et al. (2006) Cyclosporin and Timothy syndrome increase mode 2 gating of *Cav1.2* calcium channels through aberrant phosphorylation of S6 helices. *Proc Natl Acad Sci U S A* 103, 3932-3937
- 119 Muth, J. N., Varadi, G. and Schwartz, A. (2001) Use of transgenic mice to study voltage-dependent  $Ca^{2+}$  channels. *Trends Pharmacol Sci* 22, 526-532
- 120 Platzer, J., Engel, J., Schrott-Fischer, A., et al. (2000) Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type  $Ca^{2+}$  channels. *Cell* 102, 89-97
- 121 Zhang, Z., Xu, Y., Song, H., et al. (2002) Functional Roles of *Cav1.3*  $\alpha_1D$  calcium channel in sinoatrial nodes: insight gained using gene-targeted null mutant mice. *Circ Res* 90, 981-987
- 122 Sinnegger-Brauns, M. J., Hetzenauer, A., Huber, I. G., et al. (2004) Isoform-specific regulation of mood behavior and pancreatic beta cell and cardiovascular function by L-type  $Ca^{2+}$  channels. *J Clin Invest* 113, 1430-1439
- 123 Klugbauer, N., Welling, A., Specht, V., et al. (2002) L-type  $Ca^{2+}$  channels of the embryonic mouse heart. *Eur J Pharmacol* 447, 279-284
- 124 Xu, M., Welling, A., Papanisto, S., et al. (2003) Enhanced expression of L-type *Cav1.3* calcium channels in murine embryonic hearts from *Cav1.2* deficient mice. *J Biol Chem* 278, 40837-40841
- 125 Rottbauer, W., Baker, K., Wo, Z. G., et al. (2001) Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel alpha1 subunit. *Dev Cell* 1, 265-275
- 126 Moosmang, S., Schulla, V., Welling, A., et al. (2003) Dominant role of smooth muscle L-type calcium channel *Cav1.2* for blood pressure regulation. *Embo J* 22, 6027-6034
- 127 Schulla, V., Renstrom, E., Feil, R., et al. (2003) Impaired insulin secretion and glucose tolerance in beta cell-selective *Cav1.2*  $Ca^{2+}$  channel null mice. *Embo J* 22, 3844-3854
- 128 Vignali, S., Leiss, V., Karl, R., et al. (2006) Characterization of voltage-dependent sodium and calcium channels in mouse pancreatic A- and B-cells. *J Physiol* 572, 691-706
- 129 Wegener, J. W., Schulla, V., Lee, T. S., et al. (2004) An essential role of *Cav1.2* L-type calcium channel for urinary bladder function. *Faseb J* 18, 1159-1161
- 130 Wegener, J. W., Schulla, V., Koller, A., et al. (2006) Control of intestinal motility by the *Cav1.2* L-type calcium channel in mice. *Faseb J* 20, 1260-1262
- 131 MacDonald, P. E., De Marinis, Y. Z., Ramracheya, R., et al. (2007) A K ATP channel-dependent pathway within alpha cells regulates glucagon release from both rodent and human islets of Langerhans. *PLoS Biol* 5, e143
- 132 Takahashi, E., Ito, M., Miyamoto, N., et al. (2005) Increased glucose tolerance in N-type  $Ca^{2+}$  channel alpha(1B)-subunit gene-deficient mice. *Int J Mol Med* 15, 937-944
- 133 Anderson, M. P., Mochizuki, T., Xie, J., et al. (2005) Thalamic *Cav3.1* T-type  $Ca^{2+}$  channel plays a crucial role in stabilizing sleep. *Proc Natl Acad Sci U S A* 102, 1743-1748
- 134 Saegusa, H., Kurihara, T., Zong, S., et al. (2001) Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type  $Ca^{2+}$  channel. *PG - 2349-56. Embo J* 20
- 135 Pereverzev, A., Salehi, A., Mikhna, M., et al. (2005) The ablation of the *Cav2.3/E-type* voltage-gated  $Ca^{2+}$  channel causes a mild phenotype despite an altered glucose induced glucagon response in isolated islets of Langerhans. *Eur J Pharmacol* 511, 65-72
- 136 Saegusa, H., Kurihara, T., Zong, S., et al. (2000) Altered pain responses in mice lacking alpha 1E subunit of the voltage- dependent  $Ca^{2+}$  channel. *Proc Natl Acad Sci U S A* 97, 6132-6137
- 137 Ino, M., Yoshinaga, T., Wakamori, M., et al. (2001) Functional disorders of the sympathetic nervous system in mice lacking the alpha 1B subunit (*Cav2.2*) of N-type calcium channels. *Proc Natl Acad Sci U S A* 98, 5323-5328
- 138 Jun, K., Piedras-Renteria, E. S., Smith, S. M., et al. (1999) Ablation of P/Q-type  $Ca^{2+}$  channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)- subunit. *Proc Natl Acad Sci U S A* 96, 15245-15250
- 139 Fletcher, C. F., Tottene, A., Lennon, V. A., et al. (2001) Dystonia and cerebellar atrophy in *Cacna1a* null mice lacking P/Q calcium channel activity. *Faseb J* 15, 1288-90
- 140 Kim, D., Song, I., Keum, S., et al. (2001) Lack of the burst firing of thalamo-cortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type  $Ca^{2+}$  channels. *Neuron* 31, 35-45
- 141 Mangoni, M. E., Traboulsie, A., Leoni, A.-L., et al. (2006) Bradycardia and Slowing of the Atrioventricular Conduction in Mice Lacking *Cav3.1/alpha 1G* T-Type Calcium Channels. *Circ Res* 98, 1422-1430
- 142 Chen, C. C., Lamping, K. G., Nuno, D. W., et al. (2003) Abnormal coronary function in mice deficient in alpha1H T-type  $Ca^{2+}$  channels. *Science* 302, 1416-1418
- 143 Gregg, R. G., Messing, A., Strube, C., et al. (1996) Absence of the beta subunit (*cchb1*) of the skeletal muscle dihydropyridine receptor alters expression of the alpha 1 subunit and eliminates excitation-contraction coupling. *Proc Natl Acad Sci U S A* 93, 13961-13966
- 144 Weissgerber, P., Held, B., Bloch, W., et al. (2006) Reduced cardiac L-type  $Ca^{2+}$  current in *Cav3.2*<sup>-/-</sup> embryos impairs cardiac development and contraction with secondary defects in vascular maturation. *Circ Res* 99, 749-757
- 145 Namkung, Y., Smith, S. M., Lee, S. B., et al. (1998) Targeted disruption of the  $Ca^{2+}$  channel beta3 subunit reduces N- and L-type  $Ca^{2+}$  channel activity and alters the voltage-dependent activation of P/Q-type  $Ca^{2+}$  channels in neurons. *Proc Natl Acad Sci U S A* 95, 12010-12015
- 146 Freise, D., Held, B., Wissenbach, U., et al. (2000) Absence of the gamma subunit of the skeletal muscle dihydropyridine receptor increases L-type  $Ca^{2+}$  currents and alters channel inactivation properties. *J Biol Chem* 275, 14476-14481

## Voltage Dependent Calcium Channels / Products

<i>Selective Activators and Blockers</i>		
<b>Cat. No.</b>	<b>Product</b>	<b>Category</b>
BN0100	(RS)-BAY K 8644	Selective Ca <sup>2+</sup> channel (L-type) activator
BN0101	(S)-(-)-BAY K 8644	Selective Ca <sup>2+</sup> channel (L-type) activator, more active enantiomer
BN0213	FPL 64176	Potent, selective Ca <sup>2+</sup> channel (L-type) activator
BP0022	ω-Agatoxin IVa	Potent, selective Ca <sup>2+</sup> channel (P-type) blocker
BG0034	Amlodipine	Ca <sup>2+</sup> channel (L-type) blocker
BN0099	(R)-(+)-BAY K 8644	Ca <sup>2+</sup> channel (L-type) blocker, opposite effect of S-enantiomer
BP0079	ω-Conotoxin GVIA	Ca <sup>2+</sup> channel (N-type) blocker
BP0080	ω-Conotoxin MVIIC	Ca <sup>2+</sup> channel (N, P,Q-type) blocker
BG0366	Diltiazem hydrochloride	Ca <sup>2+</sup> channel (L-type) blocker
BG0188	Felodipine	Ca <sup>2+</sup> channel (L-type) blocker
BG0371	Isradipine	Ca <sup>2+</sup> channel (L-type) blocker
BG0474	Lacidipine	Ca <sup>2+</sup> channel (L-type) blocker
BG0266	Nicardipine hydrochloride	Ca <sup>2+</sup> channel (L-type) blocker
BG0268	Nifedipine	Ca <sup>2+</sup> channel (L-type) blocker, prototypic 1,4-Dihydropyridine type
BG0269	Nimodipine	Ca <sup>2+</sup> channel (L-type) blocker
BG0270	Nisoldipine	Ca <sup>2+</sup> channel (L-type) blocker
BG0271	Nitrendipine	Ca <sup>2+</sup> channel (L-type) blocker
BP0376	SNX 482	Potent, selective Ca <sup>2+</sup> channel (R-type) blocker
BN0504	SR 33805 oxalate	Potent Ca <sup>2+</sup> channel (L-type) blocker
BG0353	Verapamil hydrochloride	Ca <sup>2+</sup> channel (L-type) blocker

### Non-selective and other

<b>Cat. No.</b>	<b>Product</b>	<b>Category</b>
BG0040	Amrinone	Ca <sup>2+</sup> channel activator, PDE inhibitor
BG0452	Cilnidipine	Dual L-/N-type calcium channel blocker
BG0195	Flunarizine dihydrochloride	Ca <sup>2+</sup> channel (T-type) blocker, Na channel blocker
BN0215	Gabapentin	Binds to Ca <sup>2+</sup> $\alpha_2\delta$ subunit, anticonvulsant, increases brain GABA
BG0240	Loperamide hydrochloride	Ca <sup>2+</sup> channel blocker, opioid agonist
BS0170	Ruthenium Red	Non-selective Ca <sup>2+</sup> channel blocker, mitochondrial Ca <sup>2+</sup> blocker
BN0491	SKF 96365 hydrochloride	Calcium entry blocker
BN0686	Zonisamide	Anticonvulsant, voltage-sensitive Na <sup>+</sup> and T-type Ca <sup>2+</sup> channel blocker

### Related Radioligands

<b>Cat. No.</b>	<b>Product</b>	<b>Category</b>
ART-0462	[ <sup>3</sup> H]-Emopamil	Ca <sup>2+</sup> channel (L-type) blocker
ART-1303	[ <sup>3</sup> H]-Gabapentin	Binds to Ca <sup>2+</sup> $\alpha_2\delta$ subunit, anticonvulsant, increases brain GABA
ART-0667	[ <sup>3</sup> H]-Verapamil hydrochloride	Ca <sup>2+</sup> channel (L-type) blocker

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