

Calcium channels - basic aspects of their structure, function and gene encoding; anesthetic action on the channels - a review

[Revue : notions de base sur la structure, la fonction et l'encodage génétique des canaux calciques et action des anesthésiques sur ces canaux]

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Purpose: To review recent findings concerning Ca^{2+} channel sub-type/structure/function from electrophysiological and molecular biological studies and to explain Ca^{2+} channel diseases and the actions of anesthetics on Ca^{2+} channels.

Source: The information was obtained from articles published recently and from our published work.

Principal findings: Voltage-dependent Ca^{2+} channels serve as one of the important mechanisms for Ca^{2+} influx into the cells, enabling the regulation of intracellular concentration of free Ca^{2+} . Recent advances both in electrophysiology and in molecular biology have made it possible to observe channel activity directly and to investigate channel functions at molecular levels. The Ca^{2+} channel can be divided into subtypes according to electrophysiological characteristics, and each subtype has its own gene. The L-type Ca^{2+} channel is the target of a large number of clinically important drugs, especially dihydropyridines, and binding sites of Ca^{2+} antagonists have been clarified. The effects of various kinds of anesthetics in a variety of cell types have been demonstrated, and some clinical effects of anesthetics can be explained by the effects on Ca^{2+} channels. It has recently become apparent that some hereditary diseases such as hypokalemic periodic paralysis result from calcium channelopathies.

Conclusion: Recent advances both in electrophysiology and in molecular biology have made it possible to clarify the Ca^{2+} channel structures, functions, genes, and the anesthetic actions on the channels in detail. The effects of anesthetics on the Ca^{2+} channels either of patients with hereditary channelopathies or using gene mutation techniques are left to be discovered.

Objectif : Passer en revue les découvertes récentes concernant le sous-type, la structure et la fonction du canal Ca^{2+} à partir des études électrophysiologiques et biologiques moléculaires et expliquer les lésions des canaux Ca^{2+} et les actions des anesthésiques sur ces canaux.

Source : L'information provient d'articles publiés récemment et de nos travaux publiés.

Constatations principales : Les canaux Ca^{2+} voltage-dépendants sont l'un des importants mécanismes pour le flux entrant de Ca^{2+} dans les cellules, ce qui facilite la régulation de la concentration intracellulaire de Ca^{2+} libre. Les progrès récents en électrophysiologie et en biologie moléculaire ont permis d'observer directement l'activité des canaux et d'étudier leurs fonctions au niveau moléculaire. Les canaux Ca^{2+} peuvent être divisés en sous-types selon les caractéristiques électrophysiologiques et chaque sous-type a son propre gène. Le type L de canal Ca^{2+} est la cible d'un grand nombre de médicaments importants en clinique, spécialement les dihydropyridines, et les antagonistes des sites de liaison du Ca^{2+} ont été mis en évidence. Les effets de différentes classes d'anesthésiques dans une diversité de types de cellules ont été démontrés et certains effets cliniques des anesthésiques peuvent être expliqués par les effets sur les canaux Ca^{2+} . Récemment, il est devenu clair que certaines affections héréditaires, comme la paralysie périodique hypokaliémique, résulte de pathologies des canaux calciques.

Conclusion : Les découvertes récentes en électrophysiologie et en biologie moléculaire ont permis de clarifier les structures, fonctions et gènes des canaux Ca^{2+} et de fournir des détails sur les actions des anesthésiques sur les canaux. Il reste à découvrir les effets des anesthésiques sur les canaux Ca^{2+} des patients atteints de pathologies héréditaires des canaux calciques ou à utiliser des techniques de mutation génétique.

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IN all types of cells, the calcium ion (Ca^{2+}) is an important second messenger for intracellular signal transduction,¹ while a high concentration of free Ca^{2+} can also be a cellular toxin as has been demonstrated in ischemic brain damage. The intracellular concentration of free Ca^{2+} ($[\text{Ca}^{2+}]_i$) is, therefore, precisely controlled at a low level,^{1,2} and each cell/tissue/organ can express its function such as secretion and contraction, leading to memory, movement, pain, etc. In a resting condition, $[\text{Ca}^{2+}]_i$ is maintained at approximately 100 nM, which is 1/10,000 of the extracellular concentration of free Ca^{2+} (approximately 1 mM). There are generally three mechanisms for maintenance of a large gradient of Ca^{2+} concentrations (Figure. 1).³ Those are 1) the cell membrane, which consists of a Ca^{2+} -impermeable lipid bilayer; 2) powerful systems to excrete Ca^{2+} outside to the extracellular space [Ca^{2+} pump (Ca^{2+} -nH⁺ATPase) and Na^+ - Ca^{2+} exchanger]; and 3) intracellular organelles such as endoplasmic reticulum and mitochondria for the Ca^{2+} uptake.³

Cell function is determined by the balance of activator and inhibitor mechanisms that, in turn, are mediated by receptors and channels on the surfaces of cells.^{1,2} When the cells are stimulated by some receptor agonists, $[\text{Ca}^{2+}]_i$ increases from the resting level to approximately 1 μM , and the increased Ca^{2+} binds to intracellular substrates (e.g., calmodulin or troponin), leading to modulation of cell function. Skeletal muscle is one of the most well-differentiated organs. Intracellular Ca^{2+} storing system (i.e., sarcoplasmic reticulum) is highly developed, and quick contraction can be obtained by Ca^{2+} release from the organelles. In other general cells, an increase in $[\text{Ca}^{2+}]_i$ can be obtained by 1) rapid but transient Ca^{2+} release from intracellular Ca^{2+} stores and 2) rather slow Ca^{2+} influx from the extracellular space. Evidence has shown that Ca^{2+} influx through Ca^{2+} channels from the extracellular space is especially important for regulation of $[\text{Ca}^{2+}]_i$, and this evidence is supported, clinically, by the great number of useful Ca^{2+} antagonists such as dihydropyridines (DHPs).⁴ Recent advances both in electrophysiology⁵ and in molecular biology⁶ have made it possible to observe channel activity directly and to investigate channel functions at molecular levels. Due to these advances, the function of Ca^{2+} channels, especially voltage-dependent Ca^{2+} channels (VDCCs), has been clarified in detail, some Ca^{2+} channelopathies have been described, and direct anesthetic actions on Ca^{2+} channels have been demonstrated in many kinds of cell types.

The purpose of this review is to briefly summarize recent findings concerning Ca^{2+} channel subtype/structure/function from electrophysiological and molecular biological studies and to describe Ca^{2+} channel diseases and the actions of anesthetics on Ca^{2+} channels. Since

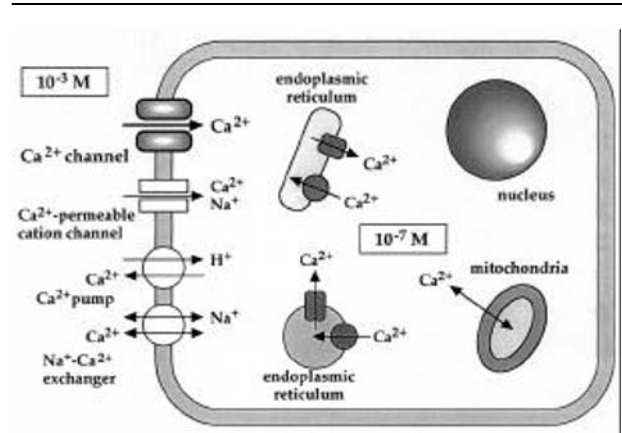


FIGURE 1 Regulation and distribution of intracellular concentration of free Ca^{2+} . The increase in intracellular concentration of free Ca^{2+} ($[\text{Ca}^{2+}]_i$) can be triggered by the release of Ca^{2+} from intracellular stores, especially the endoplasmic reticulum, and by influx from the extracellular space through voltage-dependent Ca^{2+} channels (VDCCs) and Ca^{2+} -permeable cation channels.

VDCCs serve as an important mechanism for Ca^{2+} influx into cells, leading to the regulation of $[\text{Ca}^{2+}]_i$ and cell function, we focus on VDCCs in the following sections.

Electrophysiological characteristics of voltage-dependent Ca^{2+} channel subtypes

The possible existence of VDCCs was first reported by Hagiwara *et al.*⁷ in 1975 using egg cell membrane of a starfish. They suggested that there are distinct Ca^{2+} channel types with different voltage thresholds for activation and different kinetics for opening and closing. Electrophysiological characteristics were first used to classify Ca^{2+} channels by the kinetics of opening and closing, and the conductance and lifetime of individual channels (Table I).⁸ They were initially divided into two classes: high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca^{2+} channels. The threshold activation of Ca^{2+} currents through HVA Ca^{2+} channels occurs at $-40 \sim -10$ mV, while the threshold activation of the currents through LVA channels occurs at lower membrane potentials of $-60 \sim -70$ mV. HVA Ca^{2+} channels are further divided into L-type, N-type, P/Q-type, and R-type channels, while LVA Ca^{2+} channels consists of only T-type channels. R-type is occasionally classified as intermediate-voltage-activated (IVA) channels. Ion selectivity and blocking characteristics of each channel subtype are also shown in Table I. Briefly, Ba^{2+} has an equal (T-type) or greater (others) ion selectivity over Ca^{2+} for these VDCCs. Ni^{2+} and Cd^{2+} both have a

TABLE I Electrophysiological classification and characteristics of voltage-dependent Ca^{2+} channels

	<i>T-type</i>	<i>N-type</i>	<i>L-type</i>	<i>P/Q-type</i>	<i>R-type</i>
Voltage dependence	LVA	HVA	HVA	HVA	IVA
Threshold activation (mV)	-70	-20	-30 ~ -10	-60	-40
Inactivation range (mV)	-100 ~ -60	-120 ~ -30	-60 ~ -10		
Rate of inactivation* (msec)	20-50	50-80	>500		20-40
Single channel conductance (pS)	8	13	25	10-20	14
Ion selectivity	$\text{Ba}^{2+} \approx \text{Ca}^{2+}$	$\text{Ba}^{2+} > \text{Ca}^{2+}$	$\text{Ba}^{2+} > \text{Ca}^{2+}$	$\text{Ba}^{2+} > \text{Ca}^{2+}$	$\text{Ba}^{2+} > \text{Ca}^{2+}$
Blocking by divalent ions	$\text{Ni}^{2+} > \text{Cd}^{2+}$	$\text{Cd}^{2+} > \text{Ni}^{2+}$	$\text{Cd}^{2+} > \text{Ni}^{2+}$	$\text{Ni}^{2+} > \text{Cd}^{2+}$	
Isoform	1G ¹ 1H ¹ 1I	1B	1C ¹ 1D ¹ 1S DHP	1A	1B
Antagonists/blockers	Mibefradil ω -Aga IIIA	ω -CTX GVIA ω -Aga IIIA	PAA BTZ	FTX ω -CTX-MVIIC	ω -Aga IIIA

LVA=low voltage activated; HVA=high voltage activated; IVA=intermediate voltage activated; *decay time constant; pS=picosiemens; DHP=dihydropyridine; PAA=phenylalkylamine; BTZ=benzothiazepine; ω -CTX GVIA= ω -conotoxin GVIA from conus geographus; ω -aga IIIA=agatoxin IIIA; FTX=funnel spider venom toxin; ω -CTX-MVIIC= ω -conotoxin from conus magus.

blocking effect on most of these VDCCs, and the inhibitory potency on these channels is different for each subtype.

HVA channels

1) L-type Ca^{2+} channels: the biophysical properties of L-type currents were first reported for chick dorsal root ganglion neurons in 1985,⁹ and L-type VDCCs were shown to be widely distributed in all types of cells except for platelets. In sensory neurons, L-type Ca^{2+} currents showed a slight inactivation during application of 200-msec depolarization pulses, the decay time constant being higher than 500 msec ("L"ong-lasting).¹⁰ The current begins to inactivate at holding potentials more positive than -60 mV and, when elicited from a holding potential of -60 mV, it reaches its maximum amplitude around +10 mV.

2) N-type Ca^{2+} channels: N-type Ca^{2+} channels are widely distributed in "N"eurons ("N"either L nor T). In dorsal root ganglion neurons,^{10,11} the N-type channel is distinguished by having a conductance of 13 pS, a range of inactivation between -120 and -30 mV, and a decay time constant between 50 and 80 msec. In dorsal root ganglion neurons,¹² the N-type Ca^{2+} current was isolated from other Ca^{2+} currents and recorded simply by adjusting the holding potentials and the test potentials. Although N-type Ca^{2+} currents with various biophysical characteristics have been described, the greatest variety in inactivation rates were reported with range from 100 msec in sympathetic neurons¹³ to 1.5 sec in supraoptic neurons,¹⁴ when measured in equivalent divalent ion concentrations.

3) P/Q-type Ca^{2+} channels: this channel, termed "P" because it was first described in "P"urkinje cells in 1989.¹⁵ P-type Ca^{2+} currents activate above -50 mV,

peak at around +10 mV, and display very little if any inactivation over a period of one second. In contrast to the N-type channel, the P-type channel has a unique monovalent ion selectivity in the absence of divalent cations. The sequence from the most permeable to the least is: $\text{Rb}^+ > \text{Na}^+ > \text{K}^+ > \text{Li}^+ > \text{Cs}^+$. In 1995, Randall and Tsien¹⁶ reported subsequent designations of the Q-type Ca^{2+} channel as a separate category. Because Q- (termed as the following letter of P) type current is rapidly inactivated, it requires prior elimination of P- and N-type Ca^{2+} current components before it can be studied in isolation.

LVA channels (T-type channels)

The existence of an LVA Ca^{2+} current was first discovered in 1981 in neurons of the guinea pig inferior olivary nucleus using intracellular sharp electrodes.¹⁷ T-type channels start to open with weak depolarizations reaching voltages much more negative than those required to activate other VDCCs, and the currents elicited are "T"ransient. Whole-cell recording from chick and rat dorsal root ganglion neurons has shown that the T-type current is activated at approximately -50 mV and reaches its maximum value between -40 and -10 mV. Channel inactivation is prevented at very negative potentials, while channel opening is inhibited at a holding potential more positive than -60 mV.

IVA channels (R-type channels)

R-type (termed as the following letter of Q or "R"emaining channel) currents are defined as the residual HVA Ca^{2+} current observed after the application of toxins that selectively block N-, L-, P-, and Q-type currents.¹⁸ The biophysical properties of this current are

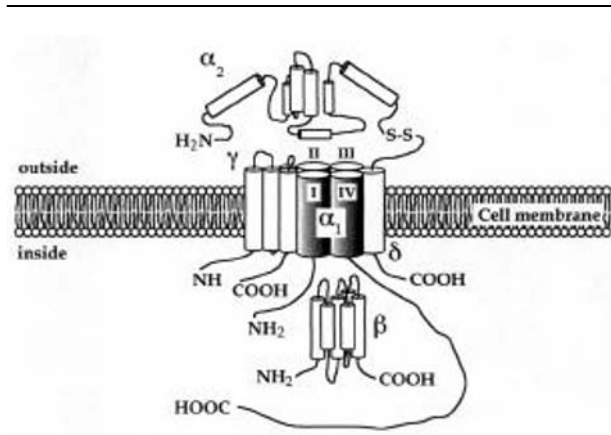


FIGURE 2 Molecular architecture of the L-type voltage-dependent Ca^{2+} channel complex. The central subunit (α_1) is made up of 24 transmembrane helices, connecting loops, and a large cytoplasmic tail. Auxiliary subunits (α_2/δ , β , and γ) surround the pore-forming α_1 subunit. The helical structures are represented by rods. Connecting loops are drawn as solid lines.

difficult to distinguish from N and Q-type currents in the whole-cell mode. There are even some parameters that are reminiscent of T-type currents (decay time constant of inactivation: 22 msec at 0 mV).

Ca^{2+} channel structure, function, and gene
Structural studies of VDCCs can explain how the channels sense changes in membrane potential and how the pores allow Ca^{2+} to pass through at a high rate while excluding other cations. Functional studies on single Ca^{2+} channels and on Ca^{2+} currents in whole cells have provided clear suggestions about the structure of the voltage sensor, the diameter of the pore, the existence of intrapore binding sites, and the presence of intracellular phosphorylation sites.

Channel structure and function

1) L-type Ca^{2+} channels: the molecular structure of the L-type Ca^{2+} channel has been widely studied in skeletal muscle because of the high concentration of L-type Ca^{2+} channels in this tissue.^{19,20} Purification and cloning of L-type channels have relied on the use of DHPs. The proposed subunit structure of the skeletal muscle Ca^{2+} channel is illustrated in Figure 2.²¹ The Ca^{2+} channel is composed of five different polypeptide subunits, having different molecular masses:²² the α_1 subunit (175 kD), which forms the ion channel and contains Ca^{2+} antagonist binding sites;^{23,24} the α_2 subunit (143 kD), which is associated with α_1 and does not contain any high-affinity binding site;²⁴ and three low-molecu-

lar-weight subunits, β (54 kD), γ (30 kD), and δ (27 kD).^{25,26} The α_1 and β subunits contain phosphorylation sites for cAMP-dependent protein kinase. The α_2 , γ , and δ subunits are heavily glycosylated, indicating that they have an extracellular face.²²

Four of the five subunits of the skeletal muscle channel have been independently cloned and sequenced: α_1 ,^{23,24} α_2 ,²⁴ β ,²⁵ and γ .²⁶ The α_1 subunit is considered to be the principal structural component of the Ca^{2+} channel. As with the Na^+ channel subunit, the Ca^{2+} channel α_1 subunit possesses four homologous domains that are predicted to span the cell membrane and to contribute to the outer vestibule of the channel pore. Each domain has six putative transmembrane regions (S1, S2...S6) (Figure 3).^{19,23,27} Both a short amino-terminal segment and a long carboxy-terminal segment of the α_1 subunit are positioned intracellularly. Studies of mutated Na^+ channels have revealed that one of the transmembrane segments, S4, serves as the voltage sensor of the channel and is present in all voltage-gated channels.²⁸ The single transmembrane segment of S4 in each motif is distinguished by a collection of repeating positively-charged amino acids (arginine or lysine), which are located in every third or fourth position. It is these four positively charged transmembrane segments that are believed to comprise the voltage sensor of VDCCs such as Na^+ and K^+ channels.²⁹

The functions of the L-type Ca^{2+} channel are related to the generation of action potentials and to signal transduction events at the cell membrane.³⁰ L-type VDCCs are expressed ubiquitously in neuronal, endocrine, cardiac, smooth, and skeletal muscle, as well as in fibroblasts and kidney cells. Recent reports suggest a role for L-type VDCCs in the process of neurotransmitter secretion of the central nervous system.^{31,32}

2) Other types of VDCCs: N-type VDCCs seem to be heterogeneous, and it is evident that they generate a current that inactivates slowly and can be mistaken for an L-type current. The N-type Ca^{2+} channel has been purified from the rat brain,³³ and a thorough purification and reconstruction study has shown that this Ca^{2+} channel purifies as a complex of four subunits with molecular weights of 230 (α_1), 140 (α_2), 95 (γ), and 57 (β) kD. The N-type VDCC plays a role in some forms of neurotransmitter release.³⁴ Direct measurement of Ca^{2+} currents from the presynaptic terminal region of the chicken ciliary ganglion calyx synapse showed that the predominant Ca^{2+} channel has pharmacological characteristics similar to those of N-type channels.³⁵

The results of immunoblotting have shown that the P/Q-type Ca^{2+} channel is composed of α_1 , α_2/δ , and β subunits.³⁶ Immunohistological studies have shown

TABLE II Voltage-dependent Ca²⁺ channel α_1 subunit genes.

<i>Isoform</i>	<i>Gene name (HUGO/GBD nomenclature)</i>	<i>Chromosomal localization</i>	<i>Tissue distribution</i>	<i>Biophysical properties</i>
<i>High-voltage activated</i>				
1A	CACNA1A	19p13.1-2	Brain, neuronal cells, heart	P-/Q-type
1B	CACNA1B	9q34	Brain, neuronal cells	N-type
1C	CACNA1C	12p13.3	Ubiquitous	L-type
1D	CACNA1D	3p14.3	Brain, neuronal cells, endocrine cells	L-type
1F	CACNA1F	Xp11.23		L-type?
1S	CACNA1S	1q31-q32	Skeletal muscle	L-type
<i>Intermediate-voltage activated</i>				
1E	CACNA1E	1q25-q31	Brain, neuronal cells	R-type
<i>Low-voltage activated</i>				
1G	CACNA1G	17q22	Brain	T-type
1H	CACNA1H	16p13.3	Kidney, liver, heart	T-type
1I	CACNA1I	22q13	Brain	T-type

that the P/Q-type channel is widely expressed in the mammalian central nervous system and that the channel appears to serve both as a generator of intrinsic activity and as a modulator of neuronal integration and transmitter release.

Because T-type VDCCs are activated at negative membrane potentials close to the resting potential, the T-type channel is thought to be responsible for neuronal oscillatory activity, which is proposed to be involved in processes such as sleep/wakefulness regulation, motor coordination, and neuronal circuit specification during ontogenesis.³⁷ In addition, T-type Ca²⁺ channels are involved in pacemaker activity, low-threshold Ca²⁺ spikes, and rebound burst firing.^{37,38} Investigation of the role of T-type channels in other systems (cardiovascular and endocrine systems in particular) has demonstrated that they are abundant in proliferating cells in both normal and pathological conditions.³⁹

Channel gene

Molecular cloning has revealed the existence in humans of at least seven different genes encoding HVA Ca²⁺ channel α_1 subunits and three LVA Ca²⁺ channel genes (Table II).⁴⁰ Analysis of sequence homology has shown among the α_1 subunit cDNAs cloned that there exists considerable base pair homology (77–90%). In addition, the finding of localization of all α_1 subunit genes on different chromosomes in humans provided further evidence that Ca²⁺ channels are encoded through a multiple gene family. As new

Ca²⁺ channel genes are cloned, it is apparent that the alphabetical nomenclatures shown in Table I will overlap at α_{1D} which may not mediate an L-type Ca²⁺ current and therefore may create confusion. For these reasons, Ca²⁺ channels should now be renamed using the chemical symbols of the principal permeating ion (Ca) with the principal physiological regulator (voltage) indicated as a subscript (Ca_v1-3).⁴¹

The α_2/δ gene has been localized to human chromosome 7q11.23-q21.1 as a single copy gene.⁴² Extensive alternative splicing has been established for the 3'-end of the message, which gives rise to the existence of five separate mRNA species.⁴³ The β genes are represented by a multigene family. cDNA cloning has established the existence of at least four genes. Thus far, alternative splicing has been shown for two β genes; therefore, the combination of a number of mature β messages may coexist in various tissues.⁴⁴ The γ subunit gene from skeletal muscle has been cloned, and the results of molecular analysis suggest that this transcript is not alternatively spliced.⁴⁵ Similar to the Na⁺ channel, the main subunit α_1 *per se* can function as Ca²⁺ channel. Other subunits (α_2/δ and β) contribute to the regulation of a Ca²⁺ channel function by changing drug affinity and/or voltage dependence.

Ca²⁺ channel antagonists and agonists

Although Ca²⁺ antagonists in a broad sense can include other types of Ca²⁺ channel antagonists such as ω -conotoxin (ω -CTX) for the N-type Ca²⁺ channel⁴⁶ and funnel spider venom toxin (FTX) for the P-

TABLE III Ca^{2+} channel antagonists and agonists (L-type)

Ca^{2+} antagonists	
(1) 1,4-DIHYDROPYRIDINES	
nifedipine	
nicardipine	
nitrendipine	
nimodipine	
nisoldipine	
felodipine	
FR34235	
PN200-110	
(2) PHENYLALKYLAMINES	
verapamil	
gallopamil	
thiapamil	
(3) BENZOTHIAZEPINES	
diltiazem	
Ca^{2+} agonists	
YC-170	
Bay K 8644	
CGP 28392	

type Ca^{2+} channel (Table I),¹⁵ Ca^{2+} antagonists in a strict sense mean L-type Ca^{2+} channel antagonists, especially DHPs.

L-type Ca^{2+} channel antagonists and agonists (Table III)

The L-type Ca^{2+} channel is the target of a large number of clinically important drugs. Ca^{2+} antagonists are classified as class IV antiarrhythmic drugs according to the Vaughan-Williams Classification.⁴⁷ The major classes of these drugs are DHPs, phenylalkylamines (PAAs), and benzothiazepines (BTZs), and these compounds are quite different chemically, biochemically, and pharmacologically.

At least six binding sites are believed to exist on the α_1 subunit for various kinds of L-type Ca^{2+} channel antagonists. As shown in Figure 3, the binding sites of the three major types of Ca^{2+} channel antagonist are different.⁴⁸ Photoaffinity labelling and peptide mapping studies of the skeletal muscle channel have revealed that all three classes bind to the transmembrane region of repeat IV of the α_1 subunit with additional sites on repeat III and repeat I for DHPs.⁴⁹ Three to four amino acids of the IV-S6 segment interact with various Ca^{2+} channel blockers. DHPs block the native and the expressed cardiac L-type Ca^{2+} channels, with nM affinity. The affinity for inhibitory DHPs increases at a depolarized membrane potential, indicating that the voltage-inactivated channel contains the conformation of the high-affinity binding site.

PAAs such as verapamil, gallopamil or thiapamil block the L-type Ca^{2+} current in a use-dependent

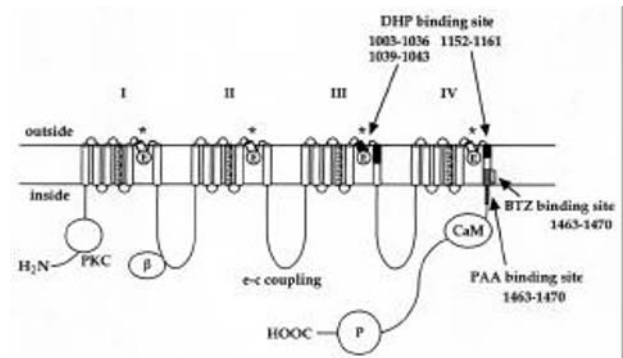


FIGURE 3 Identified structural domains of the α_1 subunit of the L-type Ca^{2+} channel. I, II, III, and IV, respective repeats of the α_1 subunit. E=glutamate; P=phosphorylation site of cAMP-dependent protein kinase; β = β subunit interaction site; CaM=calmodulin interaction site; e-c coupling=the II-III loop is responsible for the electrochemical coupling. The SS1-SS2 segments (marked by an asterisk in each motif) line the pore and bear the selectivity filter residues.

manner from the intracellular side of the membrane.⁵⁰ In contrast to PAAs, BTZs access the channel from the extracellular side. Amino acids in the IV-S5 segment proximal to the cytoplasmic mouth of the channel and close to the extracellular mouth of the channel participate in a state-dependent block of the channel by PAAs and BTZs, respectively.⁵¹

These three kinds of Ca^{2+} channel antagonists have different inhibitory effects on the cardiovascular system (Table IV). While nifedipine has a greater inhibitory effect on vessels than on the heart, verapamil has a greater inhibitory effect on the heart. That is why nifedipine is used as an antihypertensive drug and verapamil is used for treatment of arrhythmias such as paroxysmal supraventricular arrhythmia. There are two possibilities for the selectivity of DHPs on vessels. First, although there is approximately 95% homology in channel sequence between ventricular myocytes and vascular smooth muscle cells, the affinity of the α_1 subunit for DHPs is higher in vascular smooth muscle than in the cardiac ventricle. Second, the DHPs have a high affinity for the inactivated state of the Ca^{2+} channel, and vascular smooth muscle (resting membrane potential: -50 ~ -60 mV) has more inactivated channels than do ventricular myocytes (resting membrane potential: -90 mV).

Amlodipine⁵² and cinildipine⁵³ both have inhibitory effects on the N-type Ca^{2+} channel as well as on the L-type channel. Efonidipine has an inhibitory effect on T- and L-type Ca^{2+} channels.⁵⁴ The inhibitory

TABLE IV Inhibitory effects of three kinds of Ca²⁺ channel antagonists on the cardiovascular systems

	<i>Dilatation of vessels contractility</i>	<i>Inhibition of cardiac conduction</i>	<i>Prolongation of atrial-ventricular conduction</i>
Verapamil	++	+++	+++
Diltiazem	++	++	++
Nifedipine	+++	+	0

TABLE V Calcium channelopathies

<i>Disorders</i>	<i>Hereditary type</i>	<i>Gene name (HUGO/GBD nomenclature)</i>	<i>Chromosomal localization</i>	<i>Refs</i>
<i>Human</i>				
Hypokalemic periodic paralysis	AD	CACNLIA3 (L-type, _{1s})	1q31-32	63
Familial hemiplegic migraine	AD	CACNLIA4 (P/Q-type, _{1A})	19p13.1	64
Episodic ataxia type 2	AD	CACNLIA4 (P/Q-type, _{1A})	19p13.1	65
Spinocerebellar ataxia type 6	AD	CACNLIA4 (P/Q-type, _{1A})	19p13.1	66
Lambert-Eaton myasthenic syndrome	*1		67	
Malignant hyperthermia	AD	^{1A} ₂ / ^{1B} ₈ *2	7q	68
<i>Mice</i>				
Muscular dysgenesis (<i>mdg</i>)	AR	CACNLIA3 (L-type, _{1s})	Chr (F-G)	
Tottering and learner phenotypes (<i>tg</i>)		CACNLIA4 (P/Q-type, _{1A})		
Lethargic phenotype (<i>lh</i>)		CACNB4 (β_4)		
Stargazer (<i>stg</i>)		CACNG2 (neural-type, γ)	Chr 15	

*1=this disease is characterized by antibodies against these subunits. *2=malignant hyperthermia is also linked to mutation in the ryanodine receptor (RYR1); AD=autosomal dominant; AR=autosomal recessive.

effect on the N-type or T-type Ca²⁺ channel by these Ca²⁺ antagonists may bestow an additional clinical advantage for the treatment of hypertension, such as suppression of reflex tachycardia. Another novel Ca²⁺ channel antagonist, RWJ-22108, has been developed as a bronchoselective Ca²⁺ entry blocker with potential use as an antiasthmatic agent.⁵⁵ RWJ-22108 relaxes the precontracted airway in dogs with little effect on blood pressure when administered by aerosol. The inhibition of L-type Ca²⁺ channels in porcine tracheal smooth muscle cells by RWJ-22108 is qualitatively similar to that by nifedipine but with a greater stabilizing effect on the inactivated channel state.⁵⁶

In contrast to nifedipine-like Ca²⁺ channel antagonists, Ca²⁺ channel agonistic DHPs have vasoconstricting and positive inotropic properties.⁵⁷ Bay K 8644 has become the prototype of this class. Voltage clamp studies have revealed Ca²⁺ current increasing effects at 3×10^{-8} M Bay K 8644, while the current is reduced at 3×10^{-6} M.

Other types of Ca²⁺ channel antagonists (Table I)⁴⁰

In sympathetic neurons,⁵⁸ norepinephrine release is controlled by a Ca²⁺ channel that is sensitive to ω -CTX GVIA and is nitrendipine-resistant. Direct measurement of Ca²⁺ currents from the presynaptic

terminal region of the chicken ciliary ganglion calyx synapse showed that the predominant Ca²⁺ channel has pharmacological characteristics similar to those of the N-type channel:³⁵ it is resistant to DHPs and is sensitive to ω -CTX GVIA. In a subsequent series of experiments,^{59,60} single-channel analysis of chick sensory neurons, rat sympathetic neurons, and rat pheochromocytoma PC12 cells showed that ω -CTX GVIA blocks only the N-type component of the HVA current, leaving the L-type component unaffected.

Direct evidence for the existence of a third type of HVA Ca²⁺ channel (P-type) in the mammalian central nervous system was provided by the observation of a Ca²⁺ conductance that is inhibited by a toxin from funnel-web spider poison (FTX) but is not affected by other blockers.¹⁵ FTX blocks the Ca²⁺ conductance in cerebellar Purkinje cells and blocks synaptic transmission at the squid giant synapse without affecting the presynaptic action potential. Q-type currents are insensitive to DHPs and to ω -CTX GVIA and potentially, but not specifically, blocked by ω -CTX MVIIC.¹⁶ The Q-type current inactivates and is blocked by 100 nM ω -Aga IVA but requires prior elimination of P- and N-type Ca²⁺ current components before it can be studied in isolation.

Ni^{2+} sensitivity is not a reliable parameter by which to identify T-type currents because there is a high variability of sensitivity of different expressed α_1 subunits to Ni^{2+} and L-type channels can also be blocked by Ni^{2+} .^{3,9} New drugs, such as mibefradil, are being proposed as relatively specific T- and R-type Ca^{2+} channel blockers,⁶¹ although further experimentation is required to confirm the selectivity.

Abnormalities of Ca^{2+} channels (Ca^{2+} channelopathies)
The number of identified channelopathies is growing quickly. Their discovery and study are shedding new light on the pathophysiology of many neurological and muscular disorders. Information on the molecular genetics of Ca^{2+} channels has led to the discovery that alterations in a variety of Ca^{2+} channel protein complexes are linked to diseases in humans and mice (Table V).⁶²⁻⁶⁷

Hypokalemic periodic paralysis is an autosomal dominant skeletal muscle disorder manifested by episodic weakness associated with low serum K^+ . Genetic linkage analysis has shown that the gene is localized to chromosome 1q31-32 near a DHP receptor gene.⁶² The principal emphasis for management of anesthesia in patients with familial periodic paralysis is avoidance of events that will precipitate skeletal muscle weakness.^{68,69} Most other Ca^{2+} channelopathies include abnormalities of the P/Q-type Ca^{2+} channel. It appears that there are no reports on the anesthetic management of, or interaction between, the Ca^{2+} channelopathies resulting in familial hemiplegic migraine, episodic ataxia type 2, or spinocerebellar ataxia type 6. Lambert-Eaton myasthenic syndrome (myasthenic syndrome) is characterized by autoantibodies against α_{1A} and α_{1B} subunits.⁶⁶ Myasthenic syndrome is a rare disorder of neuromuscular transmission most often associated with carcinoma of the lung.⁷⁰ Patients with this syndrome are sensitive to the effects of both depolarizing and nondepolarizing muscle relaxants. The mechanism for this sensitivity is most likely due to a defect in the presynaptic release of acetylcholine.⁷¹

Malignant hyperthermia susceptibility is an autosomal dominant disorder of skeletal muscle that manifests as a potentially fatal hypermetabolic crisis triggered by commonly used anesthetic agents, especially halothane and succinylcholine.⁷² Abnormality of the ryanodine receptor of the sarcoplasmic reticulum, which is linked to L-type Ca^{2+} channels, seems to be the main cause of this disorder. Approximately half of these disorders seem to be due to ryanodine receptor abnormality. Volatile anesthetics, especially halothane, can activate the abnormal ryanodine recep-

tor extremely, resulting in an increase in $[\text{Ca}^{2+}]_i$ and muscle contraction. Although the causes in other cases are not known, Iles *et al.*⁶⁷ have found one other cause for the disorder (α_2/δ subunits, Table V). Indirect molecular genetic methods can be used to demonstrate the inheritance of an abnormal gene in certain family members at risk, and the caffeine-halothane contracture test is also used clinically.⁷² However, as yet, there has been no report on the effects of anesthetics on abnormal L-type Ca^{2+} channel activity in malignant hyperthermia.

Effects of anesthetics on Ca^{2+} channel activity

A variety of anesthetics have been shown to reduce Ca^{2+} currents through voltage-dependent Ca^{2+} channels in several cell types. Most of these results are consistent with the clinical effects of the anesthetics (e.g., cardiac depression and smooth muscle relaxation), and the inhibitory action of the channel is believed to be one of the mechanisms of the anesthetics, especially volatile anesthetics.

Volatile anesthetics

In the early 1980s, it was believed that volatile anesthetics inhibited Ca^{2+} entry through membrane-associated VDCCs, because the anesthetics showed inotropic effects on papillary muscle in the guinea pig⁷³ and inhibited nitrendipine binding to Ca^{2+} channels in the bovine heart.⁷⁴ Ikemoto *et al.*⁷⁵ first demonstrated in 1985 that halothane decreased inward Ca^{2+} slow currents in ventricular myocytes in rats, and then Terrar *et al.*⁷⁶ reported the inhibitory effect of halothane and isoflurane on Ca^{2+} channels of cardiac myocytes from the guinea pig ventricle. In 1991, investigators simultaneously reported direct inhibitory effects of volatile anesthetics, using patch clamp techniques, on Ca^{2+} channels in canine ventricular cells,⁷⁷ in canine cardiac Purkinje cells,⁷⁸ in clonal (GH3) pituitary cells,⁷⁹ and in rat sensory neurons.⁸⁰ Following these findings, many investigators have demonstrated inhibitory effects of volatile anesthetics in other kinds of tissues.⁸¹⁻⁸³

In general, volatile anesthetics at clinically relevant concentrations inhibit inward currents through voltage-dependent Ca^{2+} channels in a dose-dependent manner without an apparent change in the time course of activation or inactivation. Volatile anesthetics do not alter the voltage dependence of the currents. Based on the percent anesthetic concentrations in the gas phase, the order of inhibitory potencies for the currents is halothane > isoflurane/enflurane > sevoflurane. Volatile anesthetics also shifted the inactivation curve to a more negative potential, being inter-

preted as evidence for drug-induced stabilization of the inactivated state. Single channel analysis has shown that halothane decreased the likelihood of channel opening and enhanced the rate at which the channel closed and became inactivated.^{8,3}

A series of investigations has been conducted to examine the possible actions of volatile anesthetics on different types of VDCCs, and it has been shown that the activities of both L- and T-types VDCCs in cardiac Purkinje's cells appear to be approximately equally suppressed by halothane, isoflurane, and enflurane.^{7,6} In clonal (GH3) pituitary cells^{7,9} and porcine bronchial smooth muscle cells,^{8,4} however, it has been found that there were different sensitivities to the reductions in T-type and L-type VDCC activities caused by the anesthetics. It has also been reported that N-type VDCCs are insensitive to volatile anesthetics at clinically relevant concentrations.^{8,5} Recent molecular studies have revealed structural heterogeneity between VDCCs of different tissues,^{8,6} suggesting that these apparent discrepancies may result from differences in cell types and species and/or experimental conditions employed. Taking these findings into account, there seems to be some information concerning the actions of volatile anesthetics at the level of membrane-associated channels, although it remains to be determined whether the action of the anesthetics on Ca^{2+} channels is a direct effect on the channel proteins or whether it is a secondary consequence of, for example, alterations in membrane lipids.^{8,7}

Recent studies have revealed that the receptors for inhibitory neurotransmitters such as γ -aminobutyric acid (GABA) and glycine are sensitive to volatile anesthetics at clinically relevant concentrations.^{8,6} The functions of GABA_A and glycine receptors are enhanced by volatile anesthetics,^{8,8} whereas the activity of the related GABA I receptor is reduced. By using chimeric receptor constructs, Mihic *et al.*^{8,9} found that a region of 45 amino-acid residues was both necessary and sufficient for the enhancement of the receptor region. Although these observations support the idea that anesthetics exert a specific effect on these ion-channel proteins, this technique has not yet been employed in voltage-dependent Ca^{2+} channels.

Intravenous anesthetics

Although most *iv* anesthetics such as barbiturates and benzodiazepines have their own specific sites of action, these anesthetics also have inhibitory effects on VDCCs in many cell types. Ikemoto *et al.*^{7,5} also demonstrated the inhibitory effect of thiamylal on Ca^{2+} inward currents in rat ventricular cells. Propofol also has significant inhibitory effects on T- and L-type

components of the Ca^{2+} currents in cultured dorsal root ganglion neurons from chick embryos; this inhibition might play a role in cardiovascular side effects observed clinically.^{9,0} Thiopental, pentobarbital and propofol had little effect on P-type Ca^{2+} channel activity of cerebellar Purkinje neurons in rats,^{8,5} suggesting that P-type VDCCs do not play a major role in the induction of general anesthesia. Ketamine *in vitro* showed inhibitory effects on activation and inactivation of Ca^{2+} currents of ventricular myocytes in the guinea pig, leading to the direct myocardial depression.^{9,1} However, ketamine can support vascular tone and cardiac function presumably secondary to ketamine-induced catecholamine release. The *iv* anesthetics thiopental, ketamine and propofol all inhibited inward Ca^{2+} currents through L-type VDCCs of porcine tracheal smooth muscle cells,^{9,2} demonstrating a cellular effect of these anesthetics that can account for their airway smooth muscle relaxant effects. Thiopental, ketamine, and propofol showed similar effects on activation and inactivation of Ca^{2+} currents; however, the concentrations required to produce these effects appear to be substantially higher than the free concentrations observed clinically in serum.

Benzodiazepines have their own binding site on the GABA_A receptor, and the clinical effect of these drugs (e.g., sedation, amnesia and anticonvulsion) may be accounted for by these interactions.^{9,3} However, there is some evidence that benzodiazepines have inhibitory effects on the cardiovascular system^{9,4} and a relaxant effect on the airway,^{9,5} in which there are no benzodiazepine receptors. Diazepam was shown to significantly decrease the inward Ca^{2+} currents through expressed L-type VDCCs using heart mRNA purified from seven-day-old rats.^{9,6} Other investigators have also found that benzodiazepines had inhibitory effects on L-type VDCCs in canine myocardial cells,^{9,7} in canine tracheal smooth muscle cells,^{9,8} and in porcine intestinal mucosa cells.^{9,9} Even in neuronal cells,^{1,0,0} benzodiazepines have some inhibitory effect on non-GABA-mediated VDCC activity.

Local anesthetics

Molecular mechanisms of local anesthetics were reviewed in depth by Butterworth and Strichartz in 1990.^{1,0,1} It is accepted that local anesthetics inhibit voltage-dependent Na^+ current of the nerve membrane and, as a result, block nerve impulses responsible for conducting sensory information such as pain or tactile sensation. However, the depressant action of local anesthetics is not exerted selectively on the Na^+ current. Lidocaine at clinically relevant concentrations has been shown to inhibit inward Ca^{2+} currents in Helix gan-

glionic neurons¹⁰² and in frog dorsal root ganglionic cells.¹⁰³ A good correlation between local anesthetic potencies to inhibit HVA Ca^{2+} currents and their anesthetic potencies implies that the inhibition of Ca^{2+} influx through L/N-type VDCCs may contribute to spinal anesthesia. Lidocaine,¹⁰⁴ tetracaine,¹⁰⁵ and bupivacaine¹⁰⁶ also inhibit the VDCC activity of cardiac myocytes in the chick, guinea pig, and hamster, respectively. The inhibition is characterized by an increase in the time of recovery from inactivation and a negative-voltage shift of the steady-state inactivation curve. The inhibition is voltage-dependent, and the peak amplitude of the Ca^{2+} current cannot be restored to the control level by washout. The inhibition by local anesthetics of VDCCs in cardiac myocytes might contribute to local anesthetic-induced cardiodepression.¹⁰⁷

Conclusions

Intracellular free Ca^{2+} is important for regulation of cell functions. In all cells except for skeletal smooth muscle cells, increases in concentrations of intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$) can be obtained by rapid but transient Ca^{2+} release from intracellular Ca^{2+} stores and by slow Ca^{2+} influx from the extracellular space. Voltage-dependent Ca^{2+} channels (VDCCs) serve as one of the important mechanisms for Ca^{2+} influx into the cells, enabling the regulation of $[\text{Ca}^{2+}]_i$. Recent advances both in electrophysiology and in molecular biology have made it possible to observe channel activity directly and to investigate channel functions at molecular levels. The Ca^{2+} channel can be divided into subtypes according to their electrophysiological characteristics, and each subtype is encoded by its own gene. The L-type Ca^{2+} channel is the target of a large number of clinically important drugs, especially dihydropyridines, and binding sites of Ca^{2+} antagonists have been clarified. The effects of various kinds of anesthetics in a variety of cell types have been demonstrated, and a number of clinical effects of anesthetics can be explained by their effects on Ca^{2+} channels. It has recently become apparent that hereditary diseases such as hypokalemic periodic paralysis result from calcium channelopathies. However, the effects of anesthetics on the channel activity of patients with hereditary channelopathies have not yet been examined. Further studies will be required to investigate the effects of anesthetics both on VDCCs of the patients with calcium channelopathies and on VDCC subunits with mutation techniques.

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