PART I

NEURONAL VOLTAGE-GATED ION CHANNEL FUNCTIONS

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NEURONAL L-TYPE VOLTAGE-GATED CALCIUM CHANNELS

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1.1 STRUCTURE AND DISTRIBUTION

Although the L-type calcium (Ca^{2+}) channel has long been associated with cardiovascular physiology and pharmacology and the pharmacological and therapeutic effects of a structurally diverse group of blockers, notably of the 1,4-dihydropyridine family (Fleckenstein, 1983; Goldmann and Stoltefuss, 1991; Triggle, 2004, 2006) (Fig. 1.1), it is also widely distributed in the peripheral and central nervous system where its roles are being increasingly examined. The application of established calcium channel antagonists, such as nifedipine, does not appear to have dramatic neuronal effects, but application of dihydropyridine activators, such as Bay K 8644, produces profound neuronal and behavioral disturbances, indicating the potential pathological and therapeutic importance of these channels (Lipscombe et al., 2004; Striessnig et al., 2006). The apparent general lack of the effect of 1,4-dihydropyridines on transmitter release suggests that L-type channels play an unimportant role in presynaptic calcium entry coupled to transmitter release, but rather are involved in longer term events such as neuronal plasticity and the control of gene expression.

Structure, Function, and Modulation of Neuronal Voltage-Gated Ion Channels, Edited by Valentin K. Gribkoff and Leonard K. Kaczmarek

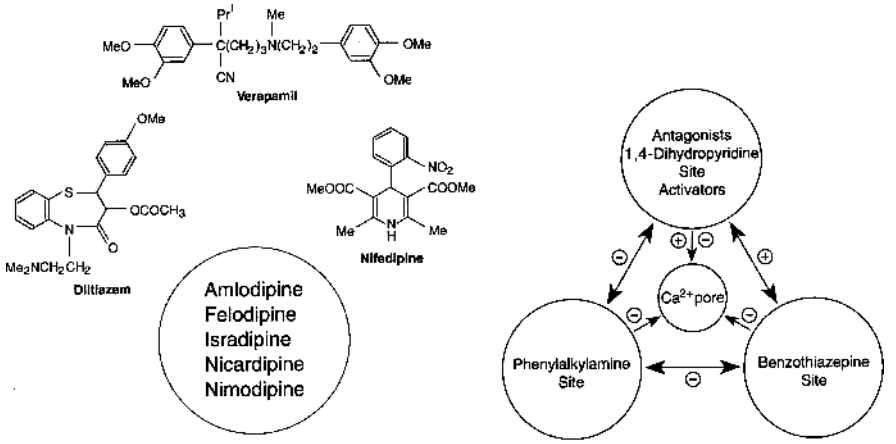


FIGURE 1.1 The structural formula and receptor site organization for the three principal drug classes active at the L-type calcium channel. A separate receptor site exists for each of the drug classes. That for 1,4-dihydropyridine activator and antagonists has been best described and explored.

However, this may be an oversimplification based in part upon an excessive reliance on the prototypical pharmacology of 1,4-dihydropyridines to define channel properties and function.

The neuronal L-type voltage-gated calcium channel (NLTCC) is a member of a family of voltage-gated calcium channels, which in turn belong to a superfamily of voltage-gated ion channels, including those for potassium (K^+) and sodium (Na^+). It is likely that the potassium channel represents an ancestral member whose functions have been progressively modified by processes of gene duplication and mutation. A number of general reviews are available for this superfamily of ion channels (McDonough, 2004; Catterall, 2005; Lacinova, 2005; Zamponi, 2005; Triggle, 2006). The voltage-gated calcium channel family is a heteromeric association of subunits as depicted in Fig. 1.2—α_1, β, $\alpha_2\delta$, and γ—and their biophysical and pharmacological properties as well as their expression are influenced significantly by the totality of the subunit interactions. Since there are several members of each subunit class with alternative splicing, the number of potential permutations with attendant variations in localization and biophysical and pharmacological properties is quite large.

1.1.1 Classification

Voltage-gated calcium channels are divided into two main classes, high voltage activated (HVA) and low voltage activated (LVA), and each of these classes is further subdivided (Catterall et al., 2005). The HVA channels are subdivided into five types (L, T, N, P/Q, and R) on the basis of their physiological and pharmacological properties. An interesting account of the history of the discovery of calcium channels has been provided by Tsien and Barrett (2005). The properties of L-type (for long-lasting) channels are summarized in Table 1.1. Figure 1.3 depicts the overall sequence

TABLE 1.1 Properties of L-Type Voltage-Gated Calcium Channels ($Ca_V1.1–1.4$)

Physiological properties	
Conductance (pS)	25
Activation threshold	High
Deactivation rate	Fast
Inactivation rate	Slow
Permeation	$Ba^{2+} > Ca^{2+}$
Function	E–C coupling, CV system, smooth muscle, endocrine cells, neurotransmitter release (eye, ear)
Pharmacological properties	
1,4-Dihydropyridines	Sensitive
Phenylalkylamines	Sensitive
Benzothiazepines	Sensitive
Benzimidazoles	Insensitive
ω-Conotoxin GV1A	Insensitive
ω-Conotoxin MVIIC	Insensitive
ω-Agatoxin IVA	Insensitive
ω-Agatoxin IIIA	Sensitive
Cd^{2+} block	Potent
Ni^{2+} block	Weak
Radioligands of choice	^{3}[H]-*cis*-(+)-Diltiazem, ^{3}[H]-desmethoxyverapamil, ^{3}[H]-isradipine
Tissue expression	Widespread: CV system, neurons, endocrine tissue, skeletal muscle, smooth muscle
Disease relevance	Hypertension, angina, malignant hyperthermia, hypokalemic periodic paralysis, night blindness

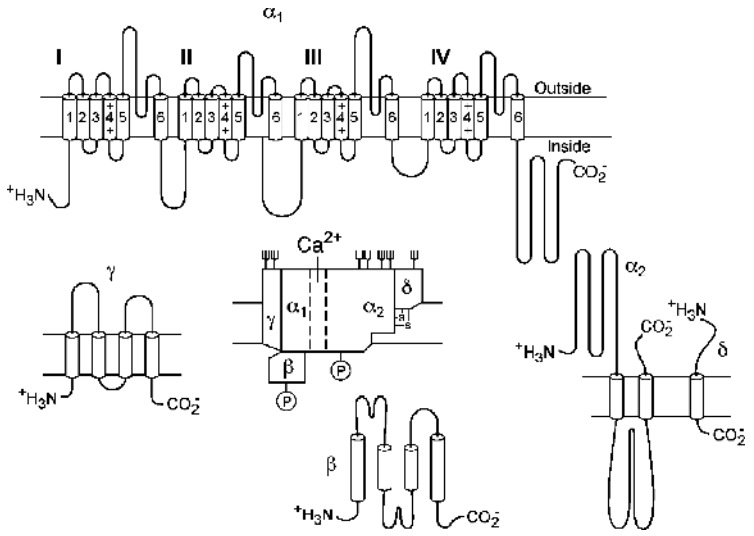


FIGURE 1.2 A schematic representation of the organization of the subunits of the voltage-gated calcium channel.

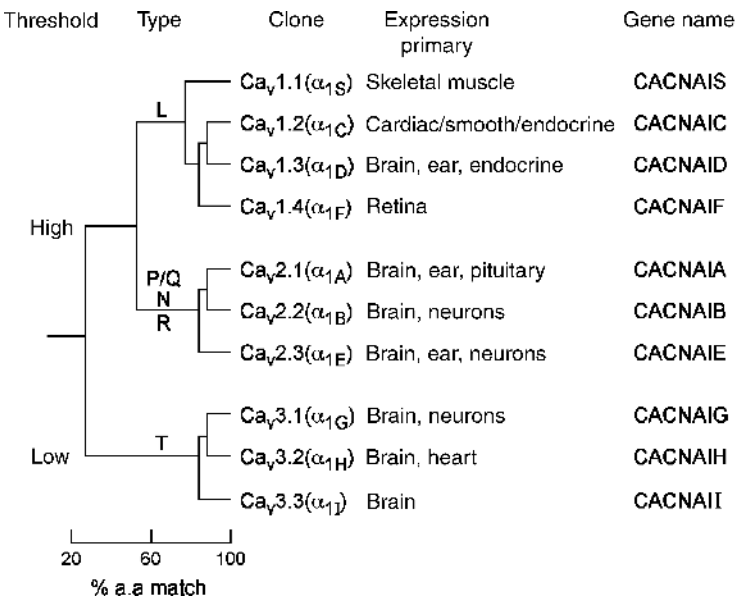


FIGURE 1.3 The organization of the voltage-gated calcium channel family.

homology between the several channel classes. The L-type voltage-gated channel, the principal focus of this chapter, belongs to the HVA class (although the potential dependence of activation and inactivation varies between subtypes) and has been particularly well investigated from structural, functional, and pharmacological perspectives. Drugs acting at this class of channel (Fig. 1.1) not only have found widespread use in the treatment of a number of cardiovascular disorders, but have also been widely explored for their potential application in a number of other disorders, including neuronal pathologies, from achalasia through depression to tinnitus and vertigo.

1.1.2 Structure

A schematic representation of the overall organization of the voltage-gated calcium channel α_1 subunit is depicted in Fig. 1.1. The α_1 subunit consists of four homologous domains, and the S4 segments contain regularly arrayed positively charged lysine and arginine residues, a feature associated with the voltage sensitivity and channel opening properties of these channels. This subunit makes up the pore-forming and voltage-sensing components of the channel as well as containing the major drug binding sites. The S5–S6 linkers each contain a critical glutamic acid residue that comprises in total the selectivity filter of the channel. A summary of the sizes of individual cloned α_1 subunits of the $Ca_v1.1$–1.4 (L-type) family is provided in Table 1.2. Interaction with other subunits is important for both expression and biophysical and pharmacological properties of the channel.

TABLE 1.2 Sizes of Cloned Ca_v1 α1 Subunits

Subunit	Origin	MW (kDa)	No. of Residues
$Ca_v1.1$	Rabbit skeletal muscle	212	1873
$Ca_v1.2$	Rabbit heart	242.8	2171
$Ca_v1.2$	Rabbit lung	242.5	2166
$Ca_v1.2$	Rat aorta	243.6	2169
$Ca_v1.3$	Human pancreas	247.6	2181
$Ca_v1.4$	Human retina	219.5	1966

Data from compilation of Lacinova (2005).

The cytosolic β subunit β_{1-4}, coded by four genes (CACNB1–4) and with a number of splice variants, interacts through specific domains on that subunit and on the S1–S2 linker: the β interaction domain (BID) of approximately 30 residues and the α interaction domain of approximately 18 residues (Pragnell et al., 1994; Hofmann et al., 1999; Van Petergem et al., 2004; Cens et al., 2005; Doering and Zamponi, 2006). The β subunits are widely distributed in excitable tissues. The β subunit resembles a membrane-associated guanylate kinase, the GK domain of which provides a hydrophobic cleft for calcium channel binding (Takahashi et al., 2004). The AID–BID complex may also provide an additional site for drug interaction (Triggle, 2004, 2006).

The $\alpha_2\delta_{1-4}$ subunit, coded by four genes CACNA2D1–D4 and with a number of splice variants, comprises two components linked by a disulfide bond with a membrane-spanning δ component and an extracellular α_2 component (De Jongh et al., 1990; Doering and Zamponi, 2006). These subunits are also widely distributed in excitable tissues. The $\alpha_2\delta_4$ subunit also generates a drug binding site, characterized in particular for drugs such as gabapentin and pregabalin used for pain relief.

The γ_{1-8} subunit is coded by eight genes, CACNG1–8, and is of particular importance in skeletal muscle ($Ca_v1.1$) and neuronal (Ca_v2 and Ca_v3) channels (Lacinova, 2005; Doering and Zamponi, 2006). The γ subunits are integral membrane proteins with four transmembrane domains and intracellular C and N termini. Of particular importance to understanding both channel function and pharmacology is the question of the actual subunit combinations that make up the various channel types: it is likely that this is tissue and cell specific and the details remain largely unresolved.

1.1.3 Subtypes

Four principal classes of the L-type channel exist represented by the α subunit of $Ca_v1.1$–1.4 and associated with the β, $\alpha_2\delta$, and particularly for the $Ca_v1.1$ class, the γ subunit. The specific permutations of association of these subunits and their splice variants remain to be fully established. However, the $Ca_v1.1$ channel, which mediates excitation–contraction coupling in skeletal muscle, is composed of the principal α_1 subunit together with β_1, $\alpha_2\delta_1$, and γ_1 subunits. The organization of the other three L channel types remains less certain, and it is possible that the subunit composition varies and is tissue dependent (Cens et al., 2005; Doering and Zamponi, 2006). In addition to the primary classification, other subtypes of the channel may exist through the

TABLE 1.3 Interaction of 1,4-Dihydropyridines with Splice Variants of the L-Type Channel

	K_D (nm), −100 mV, $Ca_V1.2a$ (Cardiac)	K_D (nm), −50 mV, $Ca_V1.2b$ (Smooth Muscle)
Nifedipine	47	10
Nisoldipine	2.1	0.56
(+)-Isradipine	15	2.1
SDZ 2017-180	91	100

Data from Morel et al. (1998).

existence of splice variants. Such subtypes may exhibit both tissue-dependent localization and tissue-selective pharmacology (Soldatov et al., 1986; Welling et al., 1993, 1997; Hu and Marban, 1998; Morel et al., 1998; Zhulke et al., 1998; Lacinova et al., 2000; Safat et al., 2001). Thus, several comparisons have been made of the properties and 1,4-dihydropyridine sensitivity of splice variants of the $Ca_v1.2$ (cardiac and smooth muscle) subunit. The a-splice variant (cardiac isoform) has a lower sensitivity to 1,4-dihydropyridines than the b-splice variant (smooth muscle). Representative data are shown in Table 1.3. Splice variants have been investigated in other tissues, including brain and neuroendocrine cells, but evidence for selective distribution and function is lacking (Safat et al., 2001).

1.1.4 Distribution

Although the dominant physiological and pharmacological expression of L-type channel activity is usually assumed to be localized to the cardiovascular system, these channels are in fact widely distributed throughout the body including the peripheral and central nervous systems (Bean, 1989; Hess, 1990; Miljanich and Ramachandran, 1995; Bech-Hansen et al., 1998; Strom et al., 1998; McRory et al., 2004; Herlitze and Mark, 2005; Lai and Jan, 2006). Within neurons, selective cellular localization occurs. $Ca_v1.4$ is present in the retina where loss-of-function mutations cause night blindness (Bech-Hansen et al., 1998; Strom et al., 1998; McRory et al., 2004). $Ca_v1.2$ and $Ca_v1.3$ are more widely distributed in nervous tissue, neuroendocrine cells, and hair cells (Herlitze and Mark, 2005). The role of $Ca_v1.3$ channels in hair cells in the cochlea is linked to the development of these cells and to the associated development of high-conductance calcium-activated potassium BK channels (Nemzou et al., 2006).

The widespread distribution of the $Ca_v1.2$ and $Ca_v1a.3$ channels within both the cardiovascular and nervous systems has made determination of their neuronal roles through pharmacological intervention difficult since the available L-type channel ligands have powerful cardiovascular properties that may overshadow or complicate any activities produced in neurons. However, selective elimination of 1,4-dihydropyridine sensitivity from $Ca_v1.2\alpha_1$ subunits permits the role of $Ca_v1.3$ channels to be examined through pharmacological dissection (Bourient et al., 2004). $Ca_v1.3$ stimulation was shown to selectively contribute to Fos expression, to neurotransmitter release in the ventral striatum, and to be associated with depression-like behavioral effects.

Within neurons, L-type channels enjoy selective localization. In rat cortex and hippocampus, $Ca_v1.2$ and $Ca_v1.3$ channels are principally localized in cell bodies and proximal dendrites, with the $Ca_v1.2$ type being concentrated in clusters and the $Ca_v1.3$ more dispersed (Hell et al., 1993; Ludwig et al., 1997). In apparent contrast, L-type channels are associated in rat globus pallidus neurons predominantly with distal dendrites, where the authors suggest that the proximal localization reported in earlier studies may represent channels in the process of transport (Hanson and Smith, 2002). The issue of the subunit association of these α_1 subunits remains unclear, but the work of Ludwig et al. (1997) on the rat brain suggests that this may be cell specific, thus providing further diversity of channel structure and function.

NLTCCs do not have a major role in neurotransmitter release but are certainly involved in the control of gene transcription activity (Dolmetsch et al., 2001; Zhang et al., 2002; Deisseroth et al., 2003; Evans and Zamponi, 2006), and the subsequent changes in protein expression may be linked to changes in synaptic strength and the regulation of transmitter phenotype (Brosenitsch et al., 1998; Deisseroth et al., 2003). Proteolytic cleavage of the $Ca_v1.2$ channel generates a C-terminal fragment, calcium channel-associated transcription regulator (CCAT), that translocates to the nucleus where it interacts with an endogenous promoter to control the expression of a number of genes associated with signaling and excitability in neurons (Gomez-Ospina et al., 2006). Other voltage-gated calcium channels behave similarly, and this may be a general control mechanism (*inter alia*, Hell et al., 1993; Westenbroek et al., 1998; Kordasiewicz et al., 2006). However, the level of such activity may differ between L-type channel subtypes: in rat hippocampal neurons, $Ca_v1.3$ plays a more important role in pCREB signaling than does $Ca_v1.2$ (Zhang et al., 2005b, 2006).

1.1.5 Mechanisms of Action: Activation, Inactivation, and Drug Action

The $Ca_v1.1$–1.4 class of channels show overall similar structure and pharmacology but differ quantitatively in a number of important aspects. The traditional view of this channel class is that they are activated by strong depolarization, are relatively slowly activated, have large single-channel conductance, show calcium-dependent inactivation, and are very sensitive to the 1,4-dihydropyridine family of ligands (Lipscombe et al., 2004). However, at least some channels formally of the $Ca_v1.2$ and 1.3 classes, those most widely distributed in the nervous system, show distinct behavior, in particular activating at relatively polarized levels of membrane potential and showing differential sensitivity to 1,4-dihydropyridines (*inter alia*, Xu and Lipscombe, 2001; Lipscombe, 2002; Lipscombe et al., 2004; Helton et al., 2005). Recombinant neuronal $Ca_v1.2$ and $Ca_v1.3$ channels open rapidly over a wide range of membrane potentials and carry significant calcium current in response to single stimulus. However, 1,4-dihydropyridines do not block calcium entry in response to single action potential stimuli but are effective in blocking current in response to step depolarization or to long trains of action potentials. This reflects the significant state-dependent interactions of 1,4-dihydropyridines (Section 1.2.2). Furthermore, 1,4-dihydropyridines completely block $Ca_v1.2$ channels, but only partially block $Ca_v1.3$ channels (Helton et al., 2005). These observations indicate that the role of neuronal L-type channel activation in response to brief stimuli may have been underestimated because of an excessive

reliance on 1,4-dihydropyridines as pharmacological markers of L-type channel activity.

1.1.6 Interaction with Other Cellular Components

In addition to interaction with β, γ, and $\alpha_2\delta$ subunits, the principal α_1 subunit of the voltage-gated calcium channel interacts with a variety of other proteins that regulate its expression, trafficking, and activity (for reviews see Catterall et al., 2005; Herlitze and Mark, 2005; Lee and Catterall, 2005; Stanley and Chan, 2005; Evans and Zamponi, 2006). Channels of the $Ca_v2.1$ and 2.2 classes interact with the βγ subunits of G proteins to mediate differential inhibition and with the SNARE protein complex that mediates exocytosis of transmitters. $Ca_v1.1$ channels interact with ryanodine receptors to mediate excitation contraction coupling in skeletal muscle and also with A kinase anchoring proteins (AKAPs) that anchor cAMP-dependent protein kinase. Ca_v1-type channels undergo both Ca^{2+}-dependent inactivation and facilitation, best established for $Ca_v1.2$ channels, mediated predominantly through calmodulin binding to the IQ-domain of the C-terminal portion of the α_1 subunit (reviewed in Lee and Catterall, 2005).

1.2 CLASSES OF DRUGS

Of the three structural classes of drugs depicted in Fig. 1.1—the benzothiazepinones, the phenylalkylamines, and the 1,4-dihydropyridines—the latter represent the largest class studied both clinically and experimentally. They are also quantitatively the most active, exhibiting pharmacology in the nanomolar concentration range, and they also include both antagonist and activator species. However, drugs active at L-type channels are not confined to these three structural scaffolds, and many diverse agents exhibit antagonist properties. The structure–function relationships of drugs active at L-type channels have been extensively reviewed over many years (*inter alia*, Janis and Triggle, 1983, 1984a, 1984b; Janis et al., 1987; Triggle et al., 1989; Goldmann and Stoltefuss, 1991; Rampe and Triggle, 1993; Triggle, 2003, 2004, 2006; Budriesi et al., 2007). Hence, only a very brief overview primarily of the 1,4-dihydropyridines, the principal therapeutic and molecular tools active at the L-type channel, will be presented here.

1.2.1 Structural Requirements

The basic structural requirements for antagonism and activation are depicted in Fig. 1.4, and the structural formulas of clinically available 1,4-dihydropyridines are depicted in Fig. 1.5. The 1,4-dihydropyridines are of particular interest for several reasons. First, they are extremely potent ligands for the L-type channel. Second, they exhibit both activator and antagonist properties. Third, they exhibit considerable stereoselectivity of action. Fourth, they show substantial state-dependent mode of interaction. Fifth, the 4-aryl-1,4-dihydropyridine nucleus is a "privileged structure" capable, when decorated with the appropriate substituents, of interacting with a variety of receptors and ion channels (Triggle, 2003).

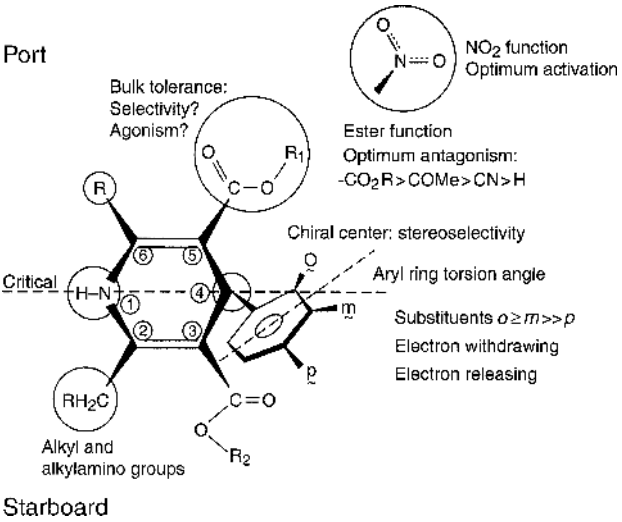
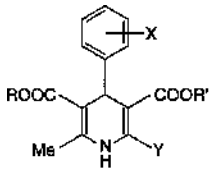


FIGURE 1.4 The structural features that determine activity of activator and antagonist in the 1,4-dihydropyridine family of drugs active at the L-type calcium channel.

Bay K 8644 serves as the prototypical activator and such compounds show highly differential pharmacology whereby one enantiomer is an activator and the other an antagonist (Fig. 1.6). Stereoselectivity is quite generally observed with all 1,4-dihydropyridines, but the extent of chirality depends on the substituents around the 1,4-dihydropyridine ring. Thus, nitrendipine has a modest stereoselectivity factor of approximately 5–10, whereas amlodipine has a (±) factor of approximately 1000.



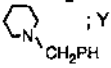
Amlodipine X=2Cl; R=Me; R'=Et; Y=$CH_2OCH_2CH_2NH_2$
Benidipine X=3NO_2; R=Me; R'=  ; Y=Me
Clinidipine X=3NO_2; R=$MeOCH_2CH_2$; R'=$CH_2CH{=}CH{\bullet}C_6H_5$(E); Y=Me
Felodipine X=2,3Cl_2; R=Me; R'=Et; Y=Me
Isradipine X=  ; R=Me; R'=$CHMe_2$; Y=Me
Lacidipine X=CH=CHCOOBu^t; R=Et; R'=Et; Y=Me
Lercanidipine X=3NO_2; R=Me; R'=$CMe_2CH_2NMeCH_2CH_2CHPh_2$; Y=Me
Manidipine X=3NO_2; R=Me; R'=CH_2CH_2N  $NCHPh_2$; Y=Me
Nicardipine X=3NO_2; R=Me; R'=CH_2NMeCH_2Ph; Y=Me
Nifedipine X=3NO_2; R=Me; R'=Me; Y=Me
Nilvadipine X=3NO_2; R=Me; R'=$CHMe_2$; Y=Me
Nimodipine X=3NO_2; R=$CHMe_2$; R'=CH_2CH_2OMe; Y=Me
Nisoldipine X=2NO_2; R=Me; R'=CH_2CHMe_2; Y=Me
Nitrendipine X=3NO_2; R=Me; R'=Et; Y=Me

FIGURE 1.5 The clinically available 1,4-dihydropyridines.

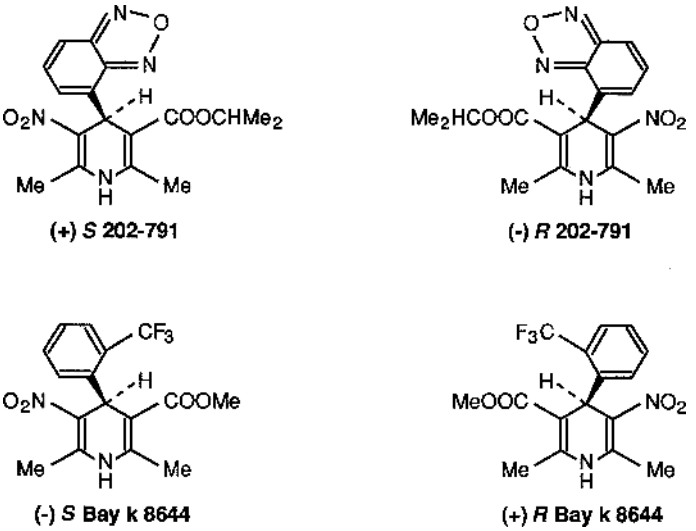


FIGURE 1.6 Stereochemical requirements for activator and antagonist activity in 1,4-dihydropyridines. The *S*-enantiomers are activators and the *R*-enantiomers are antagonists.

1.2.2 State-Dependent Actions

The 1,4-dihydropyridines (as well as verapamil and diltiazem) are characterized by their state-dependent mode of interaction at the L-type channel. These interactions arise because drugs may exhibit differential affinity and/or access to their binding sites in the resting, open, or inactivated states of the channel (Hille, 1977; Hondeghem and Katzung, 1985; Triggle, 1989; McDonough and Bean, 2006). Transitions between these states are determined by changes in chemical or electrical potential and by the kinetics of channel opening and closing. Drugs that interact preferentially with the open or inactivated state of the channel will show an increase in apparent affinity under those physiological or pathological conditions that increase the availability of those channel states. In addition, the structure of the drug and overall physicochemical properties—charged/uncharged and hydrophilic/hydrophobic characteristics—can control the access of the drug to the receptor site. Charged and polar drugs may access their binding sites through a polar and hydrophilic pathway, including the channel pore, whereas nonpolar drugs can access binding sites through membrane-delineated pathways. Both diltiazem and verapamil exhibit frequency-dependent interactions and hence their use, particularly of verapamil, in certain tachyarrhythmias, whereas nifedipine and other 1,4-dihydropyridines exhibit voltage-dependent interactions consistent with a preferential interaction with the inactivated states of the channel, a property that underscores their general vascular selectivity (Bean, 1984; Sanguinetti and Kass, 1984; Wei et al., 1986; Triggle, 1989; Zhen et al., 1992; Sun and Triggle, 1995).

1.2.3 Privileged Structures

Finally, the 4-aryl-1,4-dihydropyridine nucleus is a privileged structure and with appropriate ring substituents can access a diverse set of channels and receptors (Triggle, 2003). Certain dihydropyridines also interact with T-, N-, and P/Q-type calcium channels (Cohen et al., 1992; Kumar et al., 2002; Zhou et al., 2002; Yamamoto et al., 2006). Agents such as amlodipine, cilnidipine, barnidipine, benidipine, and nicardipine that can also block N-type channels may have some therapeutic cardiovascular advantage as well as interact at the N-type channels in neuronal tissues.

1.3 FUNCTION

1.3.1 Role of Subunits and Isoforms

The role of NLTCCs in the neuronal function is determined by the type and location of neurons as well as by the composition of channels, their subunits, and isoforms. Each channel complex consists of the pore-forming α_1 subunit and three regulatory subunits (α_2, β, and γ). Three isoforms of the α_1 subunit have been identified in the central nervous system: $Ca_v1.2\alpha_1$, $Ca_v1.3\alpha_1$, and $Ca_v1.4\alpha_1$. They have distinctly different neurological functions. The first two isoforms often occur together, expressed in the same cells, while $Ca_v1.4\alpha_1$ is found mainly in the retinal neurons (Baumann et al., 2004). The regulatory subunits also contribute to heterogeneity of NLTCCs. The $\alpha_2\delta_1$ subunit has been intensively studied and identified as a molecular target for the analgesic action of pregabalin and gabapentin (Field et al., 2006; Joshi and Taylor, 2006).

The techniques available to study physiological functions of NLTCCs are either pharmacologic or genetic. Pharmacologic methodology calls for the use of L-type Ca^{2+} channel activators or antagonists. Unfortunately, specific ligands for isoforms of the α_1 subunit of NLTCCs have not yet been discovered, and the currently available ligands for L-type channels are not isoform specific. The genetic methodology utilizes "knockout" mice lacking specific isoforms of NLTCCs (Schulla et al., 2003; Sinnegger-Brauns et al., 2004). The neurological phenotype of $Ca_v1.3\alpha_1^{-/-}$ mice exhibits inner hair cell dysfunction and cochlear sensory cell degeneration but appears neurologically normal. It has been suggested (Clark et al., 2003) that certain Ca^{2+} channel isoforms may support distinct behavioral functions. New insights into the role of NLTCCs and the $Ca_v1.3\alpha_1$ isoform in neuronal function were provided by the discovery of a link between these channels and macromolecular signaling complex formed by Shank and other modular adapter proteins as well as a link with G-protein-coupled receptors (Olson et al., 2005; Zhang et al., 2005). Using a mouse model without dihydropyridine (DHP)-sensitive $Ca_v1.2\alpha_1$ subunits ($Ca_v1.2$ $DHP^{-/-}$ mice), Sinnegger-Brauns et al. (2004) found that in the ventral striatum of these mice agonist-induced glutamate and 5-HT release was abolished, while dopamine and norepinephrine release remained intact. This observation demonstrated differences in the functions

of $Ca_v1.2\alpha_1$ and $Ca_v1.3\alpha_1$ isoforms, although both isoforms appear to control emotional behavior in mice. On the basis of experiments suitable for the dissection of function of the two isoforms, Striessnig et al. (2006) concluded that selective inhibitors of channels containing $Ca_v1.3\alpha_1$ isoforms can be expected to have antidepressant and anxiolytic properties.

1.3.2 Synaptic Plasticity and Memory

A brief period of high-frequency electrical activity applied artificially to a neuronal pathway can enhance the strength of synapses for various periods of time. This phenomenon is called long-term potentiation (LTP). It can be induced in the cerebral cortex, hippocampus, and other brain areas. Many features of LTP resemble those involved in memory storage, and LTP is widely used in memory research in attempts to elucidate molecular mechanisms of memory (Kandel, 2001). In rat hippocampus, there are at least three types of LTP (short lasting or LTP1, of intermediate duration or LTP2, and long lasting or LTP3). LTP3 is selectively dependent on NLTCCs (Raymond and Redman, 2006). In rat basolateral amygdala, LTP is induced at least in part due to influx of Ca^{2+} through channels containing $Ca_v1.2$ isoforms (Pinard et al., 2005). In the superior cervical ganglion of the rat, however, ganglionic transmission is mediated primarily by P/Q- and N-type channels with only 14% contribution by L-type calcium channels (Cifuentes et al., 2004). Antagonists of NLTCCs, for example, nimodipine, reduce the extent of LTP in rat hippocampal neurons and abolish the induction of long-term depression (LTD), induced by postsynaptic spiking prior to presynaptic activation (Bi and Poo, 1998).

The role of hippocampal NLTCCs containing $Ca_v1.2$ channels in synaptic plasticity and spatial memory was studied in $Ca_v1.2^{HCKO}$ mice in which the CACNAIC ($Ca_v1.2$) gene was inactivated (Moosmang et al., 2005b). These investigators found that the late phase of long-term potentiation (L-LTP) is lost in the hippocampus and neocortex of these animals and hippocampus-dependent spatial memory is severely impaired. A decreased activation of mitogen-activated protein kinase (MAPK) pathway and a reduced cAMP response element-dependent transcription were found in CA1 pyramidal neurons of $Ca_v1.2^{HCKO}$ mice. Phosphorylation of cAMP response element binding protein (CREB) at Ser133 is considered to be an important step in the induction of gene expression critical for memory (Moosmang et al., 2005a); it is impaired in $Ca_v1.2^{HCKO}$ mice. These observations suggest that selective inhibitors of $Ca_v1.2$ subtypes of NLTCCs could impair spatial memory.

On the contrary, there is evidence that calcineurin, the only Ca^{2+}-activated protein phosphatase in the brain, negatively modulates learning, memory, and neuronal plasticity (Mansuy, 2003). Calcineurin has been identified as the key signal in the extinction of fear memory (Lin et al., 2003). It also impairs spatial memory in mice (Mansuy et al., 1998). Ca^{2+} needed for activation of calcineurin enters neurons at least partially through NLTCCs and is likely to enhance calcineurin-induced negative modulation of learning and memory, so that inhibitors of NLTCCs could be expected to improve learning and spatial memory. It is currently unknown whether Ca^{2+} entry through channels containing $Ca_v1.2$ or $Ca_v1.3$ isoforms is linked to the activation

of calcineurin. If it is, a selective inhibitor of these isoforms could improve learning and spatial memory. The idea that antagonists of NLTCCs could improve memory and learning and be useful in the treatment of Alzheimer's disease was originally based on the calcium hypothesis of Alzheimer's disease and aging (Landfield, 1987; Disterhoft et al., 1994; Khachaturian, 1995). One of the key elements of this hypothesis involved breakdown of $[Ca^{2+}]$ homeostasis and elevation of intraneuronal calcium as a factor contributing to neuronal degeneration and death. An antagonist of NLTCCs, nimodipine, has been shown to facilitate learned behavior in rats with neocortical injury (LeVere and Sandin, 1989). More recently, nimodipine has been shown to ameliorate age-related memory decline in aged rats. This effect was associated with the decline in abnormally high level of expression of channels containing $Ca_v1.3$ subunit in the hippocampus of these animals (Veng et al., 2003). Some clinical studies suggested that nimodipine activates cognition in patients with vascular or primary degenerative dementia (Tobares et al., 1989; Fischhof et al., 1993) and another antagonist nitrendipine (Forette et al., 1998) reduces the incidence of dementia in the elderly hypertensive population. High cytosolic calcium concentrations in neurons inhibit α-secretase cleavage of amyloid precursor protein (APP) and increase intraneuronal levels of β-amyloid peptide ($A\beta_{1-42}$) (Pierrot et al., 2004). NLTCCs antagonists can be expected to reduce cytosolic calcium in neurons. Morich et al. (1996) reported clinical experience with nimodipine in patients with probable Alzheimer's disease (AD). There was no convincing evidence of effectiveness of nimodipine in AD, but this drug improved performance of patients in Buschke's Selective Reminding test, which is considered to be an index of memory storage. This finding suggests that Ca^{2+} entry through NLTCCs may modulate storage of memory in humans. The failure to demonstrate convincingly cognition activation with antagonists of NLTCCs in humans may be explained by the dual role of intraneuronal Ca^{2+}. It improves some aspects of memory but facilitates neurodegeneration and activates calcineurin, which impairs cognition. Antagonists of NLTCCs may also have effects other than those mediated by calcium channels, and these effects may oppose the consequences of calcium channel blockade. Nimodipine, for example, has been reported to stimulate $A\beta_{1-42}$ secretion in neuroblastoma cell cultures, an effect apparently not involving calcium channels (Facchinetti et al., 2006). This effect, if it occurs *in vivo*, would tend to oppose the putative cognition enhancing effect of this drug.

1.3.3 Pain

During the last two decades, substantial evidence has accumulated that voltage-gated calcium channels (VGCCs) are involved in the perception of pain (Cao, 2006; Yaksh, 2006). Pain behavior appears to be altered primarily by the $Ca_v2.2$ subunits of N-type channels, located in the presynaptic terminals where they seem to control neurotransmitter release (e.g., glutamate or substance P). The involvement of calcium channels in the control of pain was supported by the discovery of the analgesic activity of $\alpha_2\delta$ subunit ligands, gabapentin, pregabalin, and L-phenylglycine, in neuropathic pain (Taylor, 2004; Frampton and Foster, 2005; Lynch et al., 2006), although not all ligands of this subunit attenuated neuropathic pain in rat spinal cord ligation model

(Lynch et al., 2006). The $\alpha_2\delta$ protein is encoded by four genes, $\alpha_2\delta_1$, $\alpha_2\delta_2$, $\alpha_3\delta_3$, and $\alpha_2\delta_4$, and replacement of a single amino acid (arginine in a position 217) in $\alpha_2\delta_1$ subunit prevented gabapentin or pregabalin binding and nearly abolished their analgesic activity in mutant mice (Field et al., 2006). This finding strongly supports the importance of the $\alpha_2\delta_1$ subunit for the analgesic action of these two compounds. The involvement of NLTCCs in pain perception is likely to depend on the nature of the $\alpha_2\delta_1$ subunit in these channels.

Some of the early pharmacologic studies with L-type calcium channel antagonists, for example, 1,4-dihydropyridines, demonstrated their antinociceptive activity and interactions with opiates (Hoffmeister and Tettenborn, 1986; Del Pozo et al., 1987). The activity was dependent on the test, doses of the drugs, and routes of their administration, and some of the findings were contradictory. Nimodipine and nifedipine antagonized acetic acid-induced writhing following their intracerebroventricular (i.c.v.) administration to mice (Miranda et al., 1993). In the tail withdrawal test, the same drugs had antinociceptive activity in rats by chronic subcutaneous (s.c.) administration (Martin et al., 1996). In hot plate test, however, nimodipine, but not nifedipine, verapamil, or diltiazem, had analgesic effect (Miranda et al., 1992). It has been suggested that 1,4-dihydropyridines may affect pain perception at the spinal level (Martin et al., 1996). On the basis of the available evidence, L-type calcium currents appear to be only marginally involved in pain perception.

The possibility that antagonists of NLTCCs could be useful as adjuncts to analgesics has been extensively studied. Many experimental studies explored the interaction of 1,4-dihydropyridines with opiates. In the rat tail-flick test, nimodipine, at 1 mg/kg i.p., or lercanidipine, at 0.3 mg/kg i.p., potentiated analgesia caused by κ-opioid receptor agonists and prevented the development of tolerance to the opiates (Gullapalli and Ramarao, 2002a). Chronic administration of nimodipine to rats at 1 mg/kg/day i.p. for 10 days increased morphine (2 mg/kg i.p.) induced analgesia. The effect was additive to that of naloxone (Gullapalli and Ramarao, 2002b). Zhang et al. (2003) demonstrated that NLTCC antagonists inhibit morphine sensitization in mice and proposed that NLTCCs are involved in the development of morphine-induced neural and behavioral plasticity. In diabetic rats, nimodipine at 0.3–3.0 mg/kg i.p. potentiated the antinociceptve effects of morphine (Gullapalli et al., 2002). The route of administration and the duration of treatment appear to determine the ability of nimodipine to potentiate morphine. Lee and Yoburn (2000) found that nimodipine when administered s.c. to mice by a minipump at 100 μg/kg/day over 7 days, but not by single s.c. administration at 100 μg/kg, potentiated morphine-induced analgesia. This effect of nimodipine is not specific for opioids. Nociception induced by 5-HTP in mice was potentiated by nimodipine, nifedipine, or verapamil (Liang et al., 2004), and nimodipine was found to enhance the antihyperalgesic effects of diclofenac in formalin pain model in rats (Sukriti and Pandhi, 2004). In an attempt to explain the mechanism of interaction of calcium channel antagonists with morphine, Shimizu et al. (2004) pretreated mice with high doses (40–80 mg/kg i.p.) of diltiazem, nimodipine, or verapamil prior to morphine (4 mg/kg s.c.) and found that these drugs potentiate analgesic effects and increase serum levels of morphine.

By acute administration to healthy volunteers, diltiazem, nimodipine, or verapamil did not enhance the analgesic effects of morphine (Hasegawa and Zacny, 1997). In patients with cancer pain, nimodipine did not enhance morphine-induced analgesia (Roca et al., 1996). Also, in patients undergoing colorectal surgery, neither oral nifedipine nor intravenous nimodipine increased the analgesic potency of morphine (Zarauza et al., 2000). In patients undergoing knee replacement surgery, oral nimodipine increased morphine consumption without enhancing its analgesic effect (Casey et al., 2006). There is currently no definitive explanation for the apparent discrepancy between animal and human studies in respect to the ability of NLTCC antagonists to potentiate the analgesic effects of morphine. To better understand the nature of the discrepancy, the optimal blood levels of NLTCCs antagonists required for the enhancement of morphine analgesia in animals should be determined, so that the same blood levels can be achieved in clinical studies. It is conceivable that sustained release formulations of NLTCC antagonists administered for at least a week would enhance the analgesic effects of morphine.

1.3.4 Epilepsy

Epileptogenic activity in neurons is thought to be activated by an inward Ca^{2+} current. After entering neurons at least partially through NLTCCs, calcium ions are thought to regulate various aspects of synaptic activity, including epileptogenesis (De Lorenzo, 1986). The specific molecular mechanisms involved in the epileptogenesis are poorly understood, and the role of various subunits and isoforms of NLTCCs in the epileptogenic activity is not yet known. It has been suggested that antagonists of T-type calcium channels are effective in treatment of absence seizures, while NLTCCs may control partial seizures (Kułak et al., 2004). The NLTCC activator BAY K 8644 has proconvulsant activity in animals. Most antagonists of NLTCCs have been shown to have anticonvulsant activity at least in some animal models of epilepsy. Clinical data are contradictory. Anticonvulsant activity of nifedipine and nimodipine has been demonstrated in small open studies, but controlled studies with either nifedipine (Larkin et al., 1992) or nimodipine (Larkin et al., 1991; Meyer et al., 1995) were disappointing. Nimodipine has also been used in the prevention of eclampsia, but appears to be less effective than magnesium sulfate (Belfort et al., 2003).

1.3.5 Drug and Ethanol Dependence

NLTCCs appear to play a role in drug addiction and alcohol dependence, but the effectiveness of the antagonists in the treatment of drug addiction remains controversial. Nimodipine, 5–20 mg/kg s.c., or isradipine, 1–3 mg/kg s.c., inhibited self-administration of morphine or cocaine in drug-naïve mice (Kuzmin et al., 1992). At 20 mg/kg s.c., nimodipine decreased the sensitivity of rodents to the reinforcing effects of cocaine (Kuzmin et al., 1996). Cocaine-induced elevation of plasma catecholamines was prevented by nimodipine in squirrel monkeys (Trouve et al., 1990). Nitrendipine, flunarizine, or diltiazem protected rats from convulsions and death caused by a large dose of cocaine (60 mg/kg i.p.) (Trouve and Nahas, 1990).

Withdrawal signs in morphine-dependent rats were effectively antagonized by NLTCCs (Ramkumar and El-Fakahany, 1988). Chronic exposure to cocaine facilitated the function of L-type calcium channels in pyramidal neurons of medial prefrontal cortex of rats (Nasif et al., 2005). It appears that antagonists of NLTCCs may have a place in the treatment of cocaine toxicity or of withdrawal symptoms, but in cocaine-dependent patients craving for cocaine was not affected by nimodipine (Rosse et al., 1994).

Dihydropyridine-type antagonists of NLTCCs were found to interact with ethanol in animals, reversing seizures and other symptoms associated with ethanol withdrawal (Little et al., 1986; Littleton et al., 1990), blocking self-administration of 5% (but not higher concentrations) of ethanol (Smith et al., 1999), and blocking tolerance to the antinociceptive effects of ethanol during withdrawal (Gatch, 2006). Physical dependence on alcohol is associated with an increased number of dihydropyridine-sensitive calcium channels in the rat brain (Dolin et al., 1987). More recently, Katsura et al. (2005, 2006) demonstrated that physical dependence on ethanol in mice is accompanied by increased expression (and possibly function) of $Ca_v1.2$ and $Ca_v1.3$ isoforms and α_2/δ_1 subunits of NLTCCs in mouse brain. Selective antagonists of channels containing these isoforms may reduce or abolish the dependence on ethanol and possibly on other habit-forming substances.

Antagonists of NLTCCs, nimodipine, verapamil, or diltiazem, attenuated nicotine-induced locomotor sensitization and place preference in mice (Biala, 2003). The same drugs attenuated the antinociceptive effects of nicotine as well as cross-tolerance to the antinociceptive actions of nicotine and morphine (Biala and Weglinska, 2006). These findings suggest that a common mechanism involving L-type calcium channels may be responsible for the development of tolerance to the antinociceptive effects of morphine and nicotine. Chronic administration of nicotine leads to upregulation of $Ca_v1.2$, $Ca_v1.3$, $Ca_v1.4$, and $\alpha_2\delta_1$ subunits in mouse brain (Hayashida et al., 2005). Anxiogenic effects of nicotine in mice as well as the development of tolerance to this effect were attenuated by nimodipine, flunarizine, verapamil, or diltiazem (Biala and Budzynska, 2006). Attenuation of nicotine effects by NLTCCs antagonists may not, however, be mediated solely by calcium channels. It has been recently shown that nimodipine and nifedipine can also block nicotinic acetylcholine receptors (nAChRs) directly (Wheeler et al., 2006).

1.3.6 Hearing

NLTCCs and specifically $Ca_v1.3$ isoform are essential for synaptic transmission in cochlear inner hair cells and hair cell development in mice (Brandt et al., 2003, 2005; Nemzou et al., 2006). $Ca_v1.3^{-/-}$ mice are deaf and show outer hair cell loss at the apical cochlea, while heterozygous ($Ca_v1.3^{+/-}$) mice have increased hearing threshold for low-frequency sounds (Dou et al., 2004). These findings indicate the importance of the $Ca_v1.3$ subtype of NLTCCs for normal hearing. Antagonists of NLTCCs could, therefore, be expected to impair hearing. However, nimodipine improved hearing in patients with sudden hearing loss (Handrock, 1985; Theopold, 1985). In rats, it improved cochlear microphonics (Jastreboff and Brennan, 1988) and prevented

neuronal degeneration in the cochlear nerve (Sekiya et al., 2002), but did not protect gerbils from noise-induced hearing loss (Boettcher et al., 1998).

1.3.7 Vision

Light-dependent Ca^{2+} influx into photoreceptors in the retina is controlled by $Ca_v1.4$ and $Ca_v1.3$ subtypes of L-type calcium channels. These channels are located presynaptically in retinal synapses and control neurotransmitter release, primarily glutamate, but also GABA, nitric oxide, and dopamine (Barnes and Kelly, 2002). $Ca_v1.3$ is consistently expressed in AII amacrine cells, retinal neurons that have a critical role in night vision (Habermann et al., 2003). Mutations of the gene that encodes retinal $Ca_v1.4$-type channels are linked to the night blindness type 2 (CSNB2) (Hoda et al., 2006). No effect of NLTCCs on night vision has been described in the literature, but nimodipine was found to improve visual field and color vision in patients with normal tension glaucoma (Piltz et al., 1998). At 90 mg/day, nimodipine in combination with aspirin, 100 mg/day, improved visual field and hearing dysfunction in a patient with Susac's syndrome (Wildemann et al., 1996). The pathogenesis of this syndrome is unknown, but its symptomatology includes memory loss, impaired hearing, and vision loss and may conceivably involve NLTCCs.

1.3.8 Gene Transcription

The most important and critical function of NLTCCs is probably the coupling of neuronal activity to gene transcription. Nuclear transcription factors (i.e., pCREB and NFATc44) are activated by Ca^{2+} influx via postsynaptic L-type calcium channels (Bito et al., 1996; Dolmetsch et al., 2001). In hippocampal neurons, at low levels of stimulation nuclear pCREB signaling is preferentially mediated by the $Ca_v1.3$ subtype of NLTCCs (Zhang et al., 2006). The mechanism linking calcium channels to genes involves calcium channel-associated transcription regulator (CCAT). It binds to nuclear proteins and regulates the expression of endogenous genes controlling neuronal signaling and excitability (Gomez-Ospina et al., 2006). CCAT increases dendritic length and promotes contacts between neurons and extracellular matrix.

1.3.9 Cell Differentiation

Ca^{2+} influx through NLTCCs also affects expression of genes involved in cell proliferation, programmed cell death, and differentiation of neurons. According to D'Ascenzo et al. (2006), differentiation of neural stem/progenitor cells (NSCs) isolated from brain cortex of newborn mice depends on the Ca^{2+} influx through NLTCCs containing Ca_v1 isoforms. Immature GABAergic neurons are particularly sensitive to low Ca^{2+} levels, and Ca^{2+} influx through L- and T-type channels protects immature neurons from apoptosis (Pardo and Honegger, 1999). Also, in cerebellar Purkinje neurons, Ca^{2+} influx through L-type channels appears to be more important in the early rather than in the late stages of their development (Gruol et al., 2006).

1.3.10 Interactions of NLTCCs with Neurotransmitter Systems

The function of NLTCCs should not be viewed in isolation from other neurotransmitter systems. Ca^{2+} entry through NLTCCs is capable of modulating signaling of most neurotransmitter receptors, and many neurotransmitters can modulate the function of NLTCCs. Dopaminergic signaling plays a key role in the physiology and pathology of the central nervous system. D_1 receptor-mediated CREB phosphorylation depends on NLTCCs. D_1 receptor stimulation in striatal neurons reverses the effects of NLTCC antagonists on CREB phosphorylation, so that antagonists promote rather than block Ca^{2+} entry into the neurons (Eaton et al., 2004). Interdependence between dopaminergic and NMDA receptors and NLTCCs has been demonstrated (Cepeda and Levine, 1998). Cannabinoid receptor CB1 was described to inhibit calcium influx through NLTCCs in neonatal rat nucleus solitarius (Endoh, 2006), and opioids were found to modulate NLTCCs through orphan opioid receptor activation (Hurlé et al., 1999). The extent of the interplay between receptors and neurotransmitters is not yet fully appreciated and the molecular mechanisms of these interactions are not yet completely understood, but modulation of other neurotransmitter systems is likely to be an important function of not only NLTCCs but also all ion channels.

1.4 CHANNELOPATHIES AND INHERITED DISORDERS

Over the past two decades, an increasing number of mutations in voltage-gated calcium channels have been mapped and linked to a number of inherited disorders. A number of comprehensive reviews are available (*inter alia*, Lorenzo and Beam, 2000, 2005; Muth et al., 2001; Pietrobon, 2002; Striessnig et al., 2004; Pietrobon, 2005; Biduad et al., 2006; Bracey and Wray, 2006; Cannon, 2006). However, the majority of these mutations and inherited disorders are associated with non-L-type channel genes (Ca_v2 and Ca_v3), L-type channels, or associated proteins involved in muscle (skeletal, smooth, and cardiac) function. Associated with Ca_v2 and Ca_v3 genes are hemiplegic migraine, several ataxias, juvenile myocolonic epilepsy, and idiopathic generalized epilepsies, including childhood absence epilepsy. Associated with skeletal muscle are (1) hypokalemic periodic paralysis (hypoPP), a periodic muscle weakness associated with reduced serum K^+ levels and linked to missense mutations in $Ca_v1.1$ (α_{1S}); (2) malignant hyperthermia (MH) linked to multiple mutations in the ryanodine receptor associated with the voltage-sensing dihydropyridine receptor and linked to life-threatening body temperature increases during a number of pharmacological interventions, including general anesthesia and skeletal muscle relaxants; and (3) central core disease, also associated with defects in ryanodine receptors. Two cardiac muscle disorders, arrhythmogenic right ventricular cardiomyopathy and familial polymorphic ventricular tachycardia, are also linked to defects in the ryanodine receptor.

Defects in L-type channels associated with neuronal disorders have been described for $Ca_v1.2$ and particularly for $Ca_v1.4$. A mutation in the $Ca_v1.2$ gene is associated with childhood disorder termed "Timothy syndrome" associated with multiple

electrophysiological defects and sudden death caused by cardiac arrhythmias and contributed by gain of function mutations with reduced channel inactivation (Splawski et al., 2004, 2005) and aberrant phosphorylation of Ser49 in the S6 helix of domain 1 (Erxleben et al., 2006). These individuals have webbed fingers and toes (syndactyly), cognitive abnormalities, and autism. The latter pathology is of particular interest although the generalized defect produced by this mutation makes attribution to a specific neuronal pathway difficult.

By contrast, mutations in the Ca_v4 channel, almost totally distributed in and linked to retinal function (Bech-Hansen et al., 1998; Strom et al., 1998), have been well described as linked to incomplete congenital stationary night blindness (CSNB2) and characterized by varying levels of night blindness and a reduced visual acuity. Some 60 mutations have been described: approximately 50% are missense mutations leading to nonfunctional proteins, while the remainder lead to expressed channels with varying levels of dysfunction (McRory et al., 2004; Hoda et al., 2006).

It is likely that more mutations will be discovered in Ca_v1 channels linked to neuronal dysfunction, although difficulties exist because this class of channels is so widely expressed (Gargus, 2006). However, knockout studies will provide valuable leads (Muth et al., 2001). Thus, mice lacking $Ca_v1.3$ channels have both sinoatrial node dysfunction and congenital deafness, the latter being associated with degeneration of hair cells (Platzer et al., 2000; Nemzou et al., 2006). Mice lacking the $Ca_v1.2$ gene have impaired glucose tolerance and insulin secretion, the latter reflecting the absence of an exocytotic component of fusion of secretory granule attached to this channel (Schulla et al., 2003).

In addition, it is highly plausible that since the multiple cellular calcium signaling and regulatory mechanisms are closely linked, changes in one may lead to compensating changes in others. Thus, a missense mutation in the $Ca_v2.1$ channel underlies the behavior of *tottering* mice that have ataxia, paroxysmal dystonia, and spontaneous behavioral arrest (Pietrobon, 2002). Since the inducible dystonia component of this mutation can be blocked by L-type antagonists, including diltiazem, nifedipine, and verapamil, and since $Ca_v1.2$ channels are significantly upregulated in Purkinje and cerebellar cells, it appears that $Ca_v2.1$ dysfunction has produced a compensating upregulation of $Ca_v1.2$ channels (Campbell and Hess, 1999). Similarly, $Ca_v1.3$ knockout mice have impaired pancreatic islet cell function but have a compensatory overexpression of the $Ca_v1.2$ gene (Namkung et al., 2001).

1.5 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

In spite of important advances in the physiology and molecular biology of calcium channels during the past 20 years, very little progress has been made in the development of calcium channel ligands as drugs for the treatment of central nervous system diseases. No clinical use for the available activators of NLTCCs has been found. BAY K 8644 and similar activators of NLTCCs produce dystonia, self-injurious behavior, and convulsions in rodents (Jinnah et al., 2000; Kasim and Jinnah, 2003) and are unlikely to be tried in humans.

The antagonists, while widely used in cardiovascular diseases, have found only limited use in the treatment of CNS diseases (Scriabine et al., 1989, 1991; Scriabine, 2002). As noted in a recent review (Triggle, 2007), in the CNS a variety of calcium channel types contribute to neuronal activity and brain damage is likely to involve multiple pathways, so that blockade of a single channel type may not be the right strategy. Also, the failure of nimodipine in pivotal trials in either Alzheimer's disease or stroke (Mohr, 1991; Morich et al., 1996) discouraged further trials with antagonists of NLTCCs in these conditions. Subarachnoidal hemorrhage is currently the only FDA-approved indication for nimodipine in the United States. This drug is, however, prescribed "off-label'' in the prevention of migraine attacks and cluster headache in the United States and is approved for the treatment of "organic brain syndrome'' in Germany and some of the other European countries.

Further clinical trials with NLTCC antagonists in migraine as well as in drug and ethanol addiction should be considered. Such studies will probably be conducted with novel compounds, selective for subunits or isoforms of NLTCCs, or compounds with multiple sites of action. There are no selective NLTCCs yet, but Kiewert et al. (2006) described the pharmacology of NGP1-01, a polycyclic amine, which blocks NLTCCs as well as NMDA channels. Its neuroprotective potency is similar to that of nimodipine and it is structurally related to memantine, so the authors suggested its possible usefulness in stroke as well as in the treatment of neurodegenerative diseases. There is an obvious need for more basic and translational research on the function of NLTCC isoforms and their ligands. The observed interactions of NLTCCs with neurotransmitters or their receptors suggest the use of NLTCCs as adjuncts in the therapy of CNS diseases.

1.6 SUMMARY

L-type calcium channels are widely distributed in the central nervous system. Like in other tissues, the channels consist of the pore-forming α_1 and three regulatory subunits (α_2, β, and γ). Three isoforms of α_1 subunit have been identified in the central nervous system: $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$. Channels with different isoforms of the α_1 subunit differ in some of their functions. $Ca_v1.2$-containing channels appear to be involved in cognition and memory. $Ca_v1.3$-containing channels have many functions similar to those of $Ca_v1.2$-containing channels, but are essential for the synaptic transmission in cochlear inner hair cells and control hearing. $Ca_v1.4$-containing channels control Ca^{2+} influx into photoreceptors of the retina and are involved in the control of vision. L-type calcium channels are also involved in pain perception, neuronal excitability, gene transcription, and cell differentiation. They interact with other transmitter and receptor systems.

Activators of L-type calcium channels are proconvulsant and neurotoxic. Antagonists are neuroprotective but have thus far found only limited use in the treatment of CNS diseases. In the United States, only nimodipine has been approved for the prevention of neurological deficits following subarachnoidal hemorrhage. L-type calcium channel antagonists, including nimodipine, appear to be effective in

the prevention of migraine and cluster headache and can conceivably find new applications in the treatment of dementia, in drug and alcohol dependence, or as adjuncts in the treatment of epilepsy. Specific inhibitors of channels with different isoforms should be developed. Cav1.3α_1 antagonists can be expected to have antidepressant and/or anxiolytic properties.

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