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Review

The pharmacology of ion channels: with particular reference to voltage-gated Ca^{2+} channels

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Abstract

Ion channels are molecular machines that serve as principal integrating and regulatory devices for the control of cellular excitability. They are also major targets for drug action. The basic aspects of ion channel structure and pharmacological control are reviewed and illustrated with specific reference to a major class of therapeutic agents and molecular tools — the clinically available Ca^{2+} channel antagonists. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ion channel; Ca^{2+} channel; Ca^{2+} channel antagonist; State-dependent interaction; 1,4-Dihydropyridine; Verapamil; Nifedipine; Diltiazem

1. Introduction

Ion channels, although a highly heterogeneous group of membrane proteins, may be usefully regarded as a major class of pharmacological receptors with the following general properties:

- Possess drug binding sites with defined structure–function relationships, including stereoselectivity;
- Drugs exhibit agonist and antagonist properties;
- Regulated by homologous and heterologous influences, including disease state and genetically based ''ion channel diseases'' will exist;
- Should exist as homologous protein families;
- Will be associated with other cellular effector systems including cyclic nucleotide and protein kinase signaling systems.

All of these properties have been substantiated for ion channels, but such is the diversity of this class of membrane proteins that it is not possible to provide a single review of their pharmacology. Rather, this review will focus on the pharmacology of a single class of ion channels — the voltage-gated Ca^{2+} channels — that have yielded both valuable pharmacological information *and* valuable therapeutic agents and that also illustrate the general principles of ion channel pharmacology.

1.1. General principles of ion regulation through channels

Ion channels are magnificent molecular machines that play critical roles in the generation of cellular responses to a wide variety of informational inputs. Under physiologic conditions ion channels permit the orderly movement of ions across cell membranes, both plasma and intracellular. Under pathologic conditions the disorderly movement of ions through these same channels contributes to a number of disease states and cell death. Some have likened the constant movements of ions through their cellular channels as the ''music of life'' — played, of course, by the ionic ensemble. Channels permeate ions very efficiently at rates $> 10^7$ ions/s that approach diffusion controlled limits, and they do this with substantial selectivity.

Cells respond to a variety of informational inputs, both chemical and physical, and these informational inputs are coupled directly and indirectly to cellular responses through a variety of biological effectors and transduction processes. Ion channels are one of these several classes of biological effectors. Ion channels function because the cell maintains an asymmetric distribution of ions across both intracellular and plasma membranes: Na^+, Ca^{2+} and Cl^- are maintained at low intracellular levels relative to the external environment and K^+ is maintained at a high intracellular concentration (Table 1). This asymmetric distribution is

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Table 1
Ionic concentrations and equilibrium potentials[a]

Ion	$[X]_{ext}$ mM	$[X]_{int}$ mM	Equil. potential mV
Na^+	145	12	+70
K^+	5	155	−90
Ca^{++}	1.5	$<10^{-4}$	$>+120$
Cl^-	125	5	−90

[a]Average values for a range of excitable cells.

maintained because cell membranes possess selective ionic permeabilities and because of the existence of metabolically-driven ionic pumps that restore ionic gradients in the face of leakage and gradient dissipation following channel activation. Accordingly, each ionic current can be characterized by an equilibrium potential at which the *net* movement of ions will be at equilibrium. These properties have been well reviewed in standard texts (Aidley and Stanfield, 1956; Hille, 1992).

1.2. Pharmacological control of ion movements

Because of these ionic distributions the selective opening or closing of specific ion channels will have important consequences to membrane potential and cellular excitability. Quite generally, the selective activation of Na^+ or Ca^{2+} channels will depolarize the cell and have excitatory consequences, whilst the selective opening of K^+ or Cl^- channels will hyperpolarize the cell and have inhibitory consequences. There are important consequences to these considerations of ionic gradients and their control for the action of drugs. Drugs that open Na^+ or Ca^{2+} channels will be excitatory agents and drugs that open K^+ or Cl^- channels will be inhibitory in character: the opposing relationship will hold for antagonists or blockers of these same channels (Fig. 1). However, the control of ion channels by physiologic events or by drugs should not be viewed as isolated events: ion channel activities are frequently linked events, whereby the consequences of activation of one channel are the activation or inactivation of another channel type (Fig. 2).

Because ion channels serve as integrating loci for a variety of inputs, both excitatory and inhibitory, a variety of specific pharmacological interventions can achieve the same end consequence. Thus, neuroprotection may be achieved through several discrete ion channels that mediate inhibition of cellular Ca^{2+} influx and mobilization both directly or through cellular hyperpolarization and reduction of activation of voltage-gated Ca^{2+} channels (Fig. 3). Finally, each ion channel typically has a number of discrete drug binding sites, occupancy of which can regulate channel activity by competitive, allosteric, pore occupancy and other processes (Fig. 4).

1.3. Ion channel classification

In principle, ion channels may be classified by a variety of criteria — ionic selectivity, sensitivity to physiological ligands (neurotransmitters), voltage sensitivity and structure including subunit composition. Accordingly, we recognize several distinct classes or families of ion channels including most notably the ligand-gated channels (nicotinic acetylcholine, *gamma*-aminobutyric acid, glycine, $5\text{-}HT_3$ etc), and the voltage-gated ion channel family (Na^+, K^+ and Ca^{2+}). Each of these superfamilies exhibit substantial

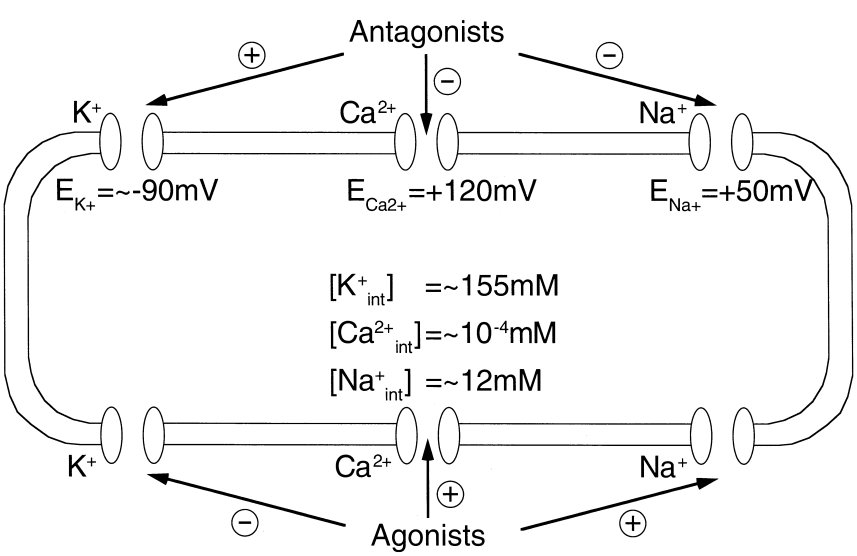

Fig. 1. The asymmetric distribution of ions in a cell is maintained by ion pumps and the selective permeability of cell membranes and an equilibrium potential exists for each ion. The opening or closing of ion-specific channels by agonists or antagonists will serve to alter the membrane potential across the cell, depolarizing (+) or hyperpolarizing (−), according to the nature of the ion channel and the drug.

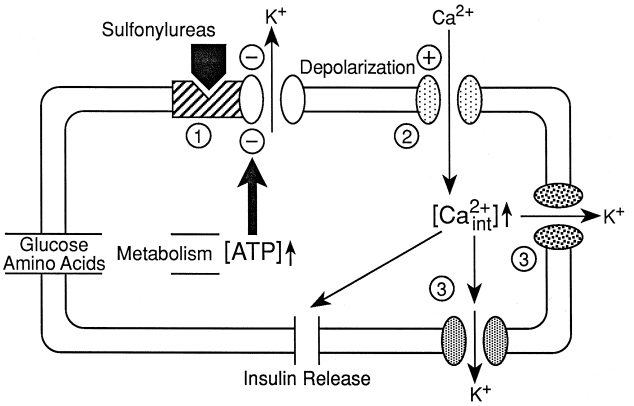

Fig. 2. Ion channels frequently act in linked manner. In the depicted pancreatic *beta*-cell elevation of ATP following metabolism blocks the ATP-sensitive K^+ channel (also blocked by the sulfonylurea anti-diabetic drugs) to depolarize the cell thus activating an L-type voltage-gated Ca^{2+} channel to permit the influx of Ca^{2+} and trigger insulin release. In turn, the elevated intracellular Ca^{2+} can activate a Ca^{2+}-sensitive K^+ channel to hyperpolarize the cell.

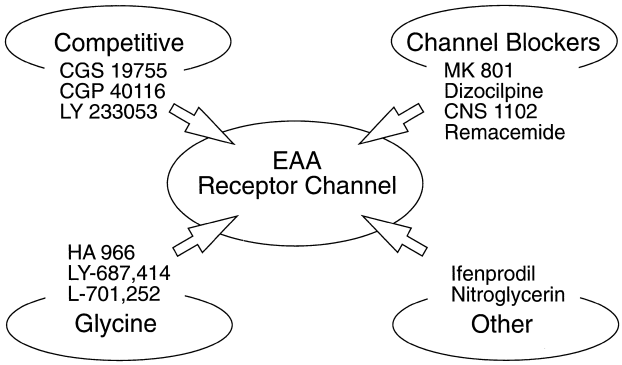

Fig. 4. A given ion channel possesses several, frequently four to ten, discrete drug binding sites for ligands that modulate channel function by independent processes. This is illustrated for the excitatory amino acid ligand-gated ion channel.

structural and functional homology, but more importantly all ion channels regardless of classification possess a signature ''pore region'' derived from the folding of transmembrane segments derived from a single major ion channel protein (Na^+, Ca^{2+}) or from the homomeric or heteromeric oligomerization of subunits (K^+, ligand-gated). (reviewed in Rampe, 1994; Catterall, 1995; Ortells and Lunt, 1995; Dolly and Parcej, 1996).

1.4. General principles of channel organization

Principally through electrophysiological analysis a model of ion channel organization has been assembled that possesses the mechanical features appropriate for function (Fig. 5). These features include sensors that are sensitive to chemical or physical signals, ''gates'' that open and close in response to these signals, a pore that selectively permeates ions and a ''selectivity'' filter that confers upon the channel its ionic discrimination capacity. The basic correctness of this model has been amply confirmed quite recently with the elucidation of the solid state structure of a K^+ channel from *Streptomyces lividans* (Armstrong, 1998; Doyle and Cabral, 1998). This structure reveals highly helical transmembrane domains in an ''inverted tepee'' directed towards the extracellular surface, and a P loop that contains a highly conserved ''signature sequence'' — Thr–Xxx–Thr–Thr–Xxx–Gly–Tyr–Gly — that plays a key role in ionic selectivity through cation–carbonyl

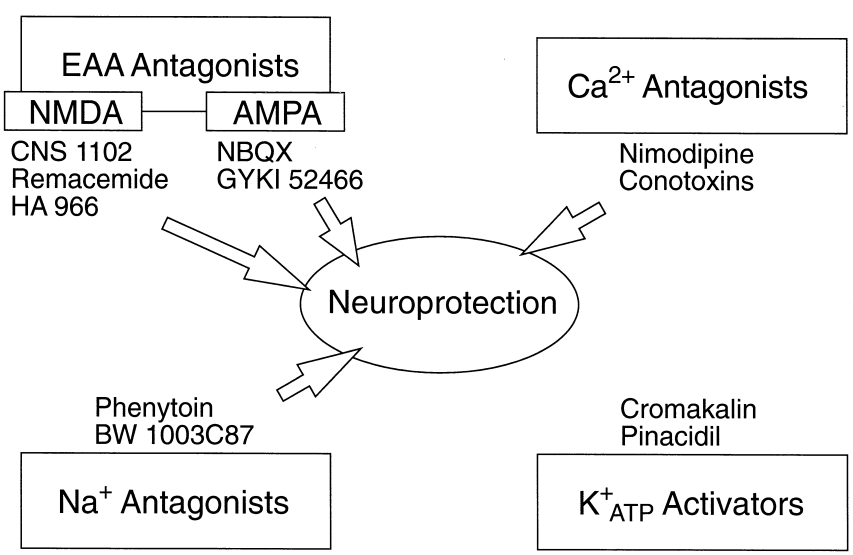

Fig. 3. A given pharmacological end point or therapeutic consequence may be achieved by several different and independent classes of ion channel drugs. In the example shown ''neuroprotection'' is, in principle, achieved by drugs that stabilize membrane potential and/or reduce neuronal Ca^{2+} mobilization and influx at several discrete channel classes, including voltage-gated Ca^{2+} channels, ligand-gated excitatory amino acid channels and receptors, voltage-gated Na^+ channels and ATP-sensitive K^+ channels.

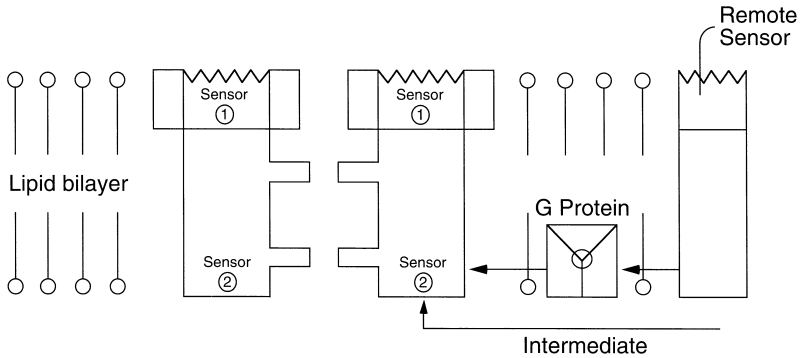

Fig. 5. A schematic model of ion channel organization depicting an ion-selective pore that constitutes a selectivity filter, ''gates'' both internal and external that regulate the opening and closing of channels and sensors that are responsive to physical and chemical signals that alter the probability of channel opening. These sensors may be remote and comprise other receptor systems that are linked via intermediate messengers to the ion channel.

interactions with multiple ion occupancy expediting ion flow (Armstrong, 1998; Armstrong and Hille, 1998; Doyle and Cabral, 1998). Since the K^+ channel is widely regarded as an ''ancestral'' ion channel this structure determination, albeit at still comparatively low resolution, is important since it is it is likely to apply, at least in broad outline, to all other channels.

1.5. Drug interaction with ion channels

The presence of drug binding sites with ion channels is well known and well described. Indeed, as with other major classes of pharmacological receptors drugs, both agonists and antagonists, provide a very useful way of classifying and sub-classifying ion channels (Fig. 6). However, there are additional and specific aspects of drug interaction with ion channels that add both complexities and subtleties to the quantitation of drug action. These additional features of drug action at ion channels arise from the conformational changes that occur as a channel passes through a series of transitions from resting to open to inactivated (Fig. 7). In principle each of these states, or families of states, offers different access opportunity or exhibits different affinity for the drug. Thus, an agent that

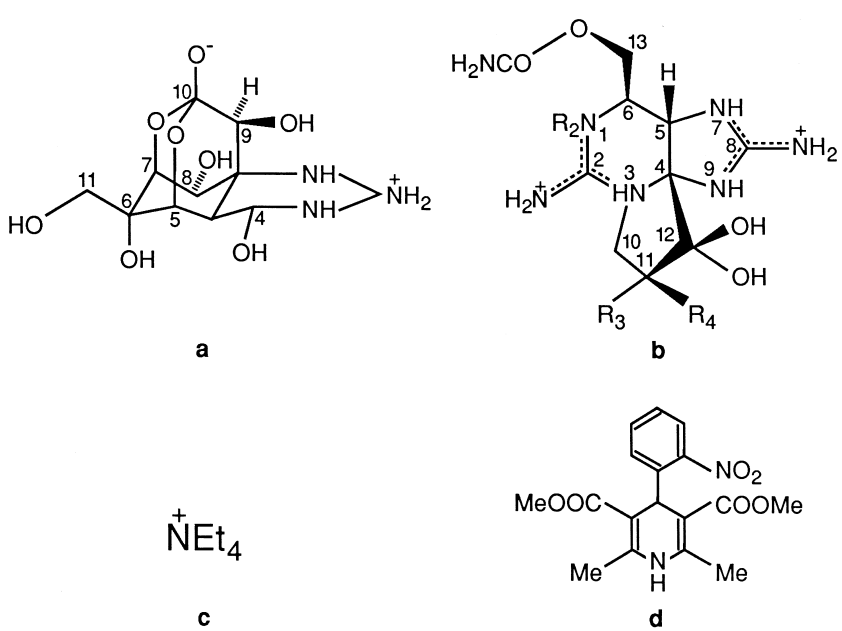

Fig. 6. Ion channels are characterizable by drugs that specifically recognize discrete channel classes: (a, b) tetrodotoxin and saxitoxin for Na^+ channels; (c) tetraethylammonium for K^+ channels; (d) nifedipine for Ca^{2+} channels. The differential sensitivity of these drugs permits also the subclassification of channels: nifedipine interacts with selectivity at the L-type of Ca^{2+} channel and tetraethylammonium, although a low affinity ligand, discriminates between voltage-gated K^+ channels.

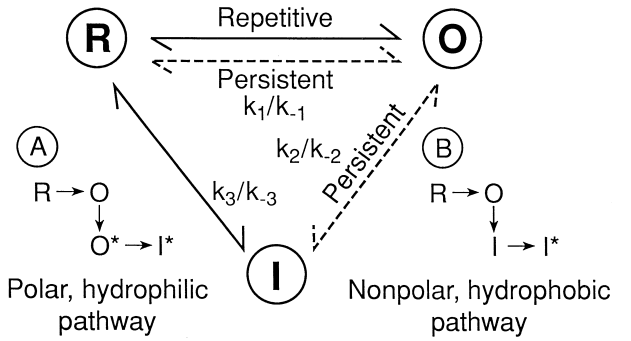

Fig. 7. A schematic representation of an ion channel cycling between resting (R), open (0) and inactivated (I) states. The equilibrium between these states will depend upon the membrane potential, the frequency and intensity of stimulus and the effects of biochemical modification of the channel. Additionally, the structure of the drug will influence access to binding sites via hydrophobic or hydrophilic pathways. The differential affinity of a drug or its differential access according to state provides the basis for the ''state-dependent'' interactions of drugs with their binding sites at ion channels.

more easily accesses its binding site when the channel is in the open state or where the binding affinity of the drug is greater in the open state will exhibit a higher pharmacological, biochemical or clinical affinity for its receptor during any condition, physiological or pathological, that increases the fraction of the channel in the open state. Such ''state-dependent binding'' provides a mechanism whereby the same drug may exhibit a continuum of affinities for the same binding site according to the state of the channel (Hille, 1977; Hondeghem and Katzung, 1985; Triggle, 1989).

According to this modulated receptor hypothesis:

- Different channel states have different affinities for drugs;
- Drugs may exhibit quantitatively and qualitatively different structure–function relationships for different channel states, including changes in stereoselectivity;
- Drugs stabilize different channel states;
- Drugs alter the kinetics of channel state interconversion.

Thus, if a channel exists in states A and B, characterized by the microscopic dissociation constants K_A and K_B, and if h is the fraction of the channel in state A, then K_{app} the apparent or measured dissociation constant is given by:

$$K_{app} = 1/(h/K_A) + (1 - h/K_B) \quad (1)$$

If K_A and K_B differ by one thousand-fold then K_{app} can, in principle, span the same range of measured affinities. The application of this hypothesis to drug action at voltage-gated ion channels has been amply confirmed for a variety of drug classes acting at different channel classes. The actions of local anesthetics and related anti-arrhythmics at Na^+ channels and quaternary ammonium ions at K^+ channels have been particularly well described by this

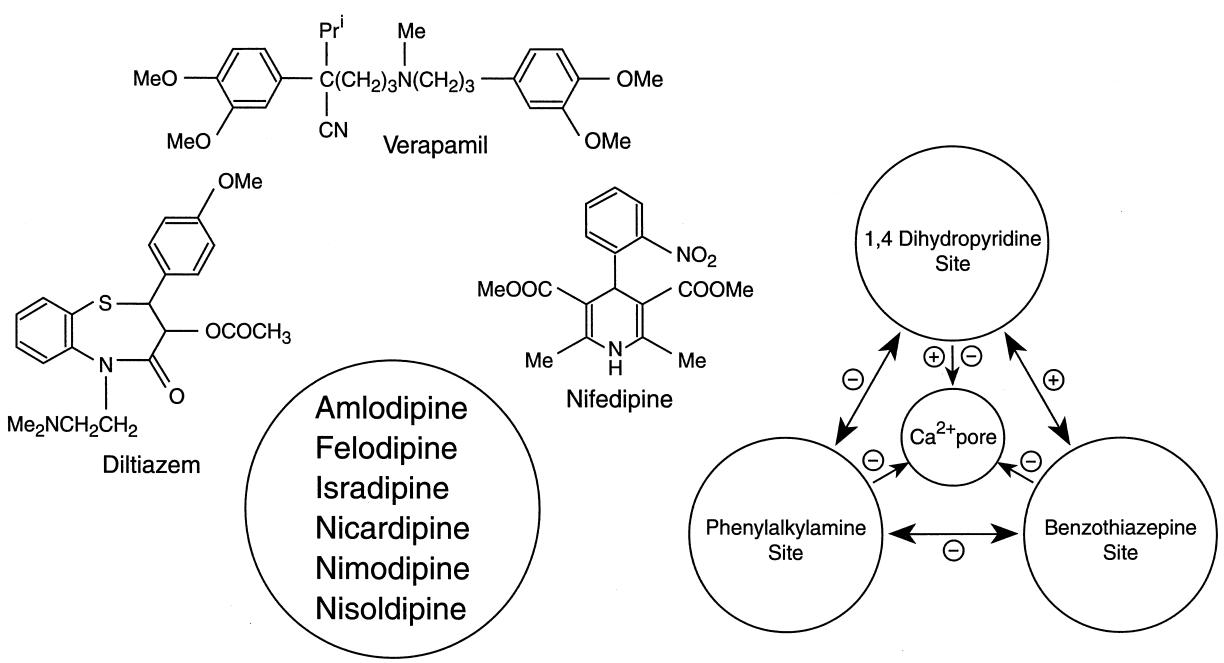

Fig. 8. The calcium channel antagonists, verapamil, nifedipine and diltiazem, interact at three discrete receptors associated with the voltage-gated L-type channel that dominates the functional properties of the cardiovascular system. These binding sites are linked one to the other by allosteric interactions that also link these sites to the functional machinery of the channel. Second generation 1,4-dihydropyridine antagonists (nifedipine class) are presumed to occupy the same 1,4-dihydropyridine receptor as does nifedipine.

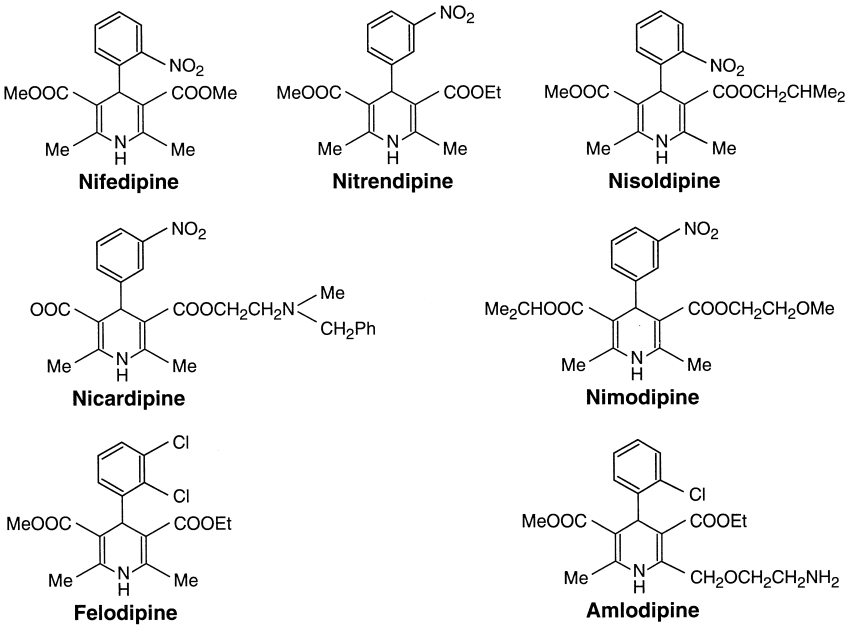

Fig. 9. 1,4-Dihydropyridine antagonists (nifedipine, nitrendipine, nicardipine, nimodipine, felodipine, isradipine and amlodipine).

hypothesis (Hille, 1977; Bean et al., 1983; Hondeghem and Katzung, 1985; Scheuer, 1999).

1.6. Ion channels as targets for drug design

Ion channels constitute very attractive targets for drug action and drug design. This has been exploited by both man and nature.

Many of the most potent and lethal toxins of nature, from both vertebrate and invertebrate species, target ion channels and therapeutic agents are available that target virtually all of the major ion channel categories. Of particular prominence in this respect are the calcium channel blockers, major cardiovascular agents, whose worldwide sales translate into the several billions of dollars.

Ion channels have several properties that make them attractive targets. These include:

- Loci for integrated cellular communications;
- Highly efficient molecular machines;
- Multiplicity of channel types;
- Multiplicity of drug binding sites;
- Modulation of drug binding by membrane and chemical potential.

These general principles of drug action at ion channels are usefully illustrated with reference to one particular channel family — the voltage-gated Ca^{2+} channels.

2. Voltage-gated Ca^{2+} channels

The discovery of the group of drugs generally referred to as ‘‘*the calcium blockers*’’ or the ‘‘*calcium channel*

Table 2
Cardiovascular profile of calcium channel antagonists

	Nifedipine	Diltiazem	Verapamil
Coronary vessels			
Tone	− − −	− −	− −
Flow	+ + +	+ +	+ +
Peripheral vasodilation	+ + +	+	+ +
Heart:			
Rate	+ +[a]	−	−
Contractility	0[b]	−	−
A–V node conduction	0	−	−
A–V node ERP	0	−	−

+ Increase; − decrease; 0 no effect.
[a]Reflex sympathetic activation.
[b]No depressant effect unless left ventricular dysfunction.

Table 3
Therapeutic application of calcium antagonists

	Verapamil	Nifedipine	Diltiazem
Angina			
Exertional	×	×	×
Unstable	×	–	×
PSVST	×	–	×
Atrial fibrillation and flutter	×	–	×
Hypertension	×	×	×
Peripheral vascular disorders (Raynaud’s)	–	×	–
Cerebral vasospasm[a] (post-hemmorhage)	–	×	–

×, Use; –, not used.
[a]Role for Nimodipine.

Table 4
Side effects of calcium channel antagonists

	Diltiazem	Nifedipine	Verapamil
Ankle edema	+ +	+ +	+ +
Constipation	+	0	+ + +
Dizziness	+ +	+ +	+ +
Facial flushing	+	+ + +	+ +
Headaches	+	+ +	+
Ischemia	0	+	0
Rash	+	0	+
Tachycardia	0	+ +	0

+, + +, + + + — Degree of use; 0, not used.

Table 6
Cardiovascular selectivity of calcium antagonists in human tissue

Antagonist	Ratio IC_{50} Heart/ IC_{50} Vessel
Diltiazem	~1
Verapamil	~1
Nifedipine	~10
Amlodipine	~10
Felodipine, Isradipine, Nifedipine, Nicardipine, Lacidipine	~100
Nisoldipine	~1000

Data from Godfraind et al. (1992).

antagonists'' owes much to a century-long development of the understanding of the key role of Ca^{2+} as a cellular messenger *par excellence* and of the multiple pathways through which the cell expends its energy and creativity in the regulation of the storage and mobilization of this cation. Early and critical players in this understanding were Ringer and Heilbrunn (Ringer, 1883; Heilbrunn, 1943). The typical cell probably possesses and uses some 5–10 calcium regulatory processes and each of these sites and pathways is, in principle, subject to regulation by specific drugs.

In practice, the only drugs thus far therapeutically available are the calcium channel antagonists, defined by the prototypical first generation, verapamil, nifedipine and diltiazem (Fig. 8). These are well recognized to be chemically heterogeneous and their discovery and classification owes much to the work and tenacity of Albrecht Fleckenstein and his colleagues (reviewed in Fleckenstein, 1983). Their work showed that these agents produced an effective electromechanical uncoupling in cardiac tissue and that these effects mimicked those of calcium withdrawal. Similarly, the work of Godfraind and his colleagues (Godfraind et al., 1992) in vascular smooth muscle established that diphenylpiperazines of the cinnarizine and flunarizine class effectively blocked contraction in depolarized vascular smooth muscle and that, *''la cinnarizine est un antagonist du calcium, au niveau de muscle vasculaire depolarise''* (Godfraind and Polster, 1968).

Table 5
Contraindications of calcium channel antagonists

	Diltiazem	Nifedipine	Verapamil
Aortic Stenosis	+	+	+
AV conduction defects	+	0	+
Cardiac failure	+	+	+
Hypertension	+	+	+
Sinus bradycardia	0, +	0	0, +
Sick sinus syndrome	+	0	+

+, Contraindication; 0, no contraindication.

2.1. Basic pharmacology and therapeutic applications

The calcium channel antagonists are a chemically, pharmacologically and therapeutically heterogeneous group of drugs that are not simply interchangeable one for the other, though they have a common target the voltage-gated Ca^{2+} channel. This distinctiveness of action also applies to the second-generation agents which are all of the 1,4-dihydropyridine class and include amlodipine, felodipine, nimodipine, nicardipine and nitrendipine (Fig. 9).

Table 2 reveals that these agents differ in their relative actions on cardiovascular tissues. Verapamil, nifedipine and diltiazem are all vasodilating drugs, although nifedipine and all other available 1,4-dihydropyridines are quantitatively the more potent. However, verapamil and diltiazem have significant cardiac depressant properties and these underlie their role as Class IV anti-arrhythmic agents. Quite generally verapamil and diltiazem are regarded as ''non-selective'' blockers while nifedipine and other 1,4-dihydropyridines are described as ''vascular-selective'', although their degree of vascular selectivity varies considerably among 1,4-dihydropyridines. These activities define

Table 7
Ca^{2+} channel antagonist classification

	Vasodilatation	Contractility	A-V Conduction
Verapamil	++	+++	+++
Diltiazem	++	++	++
Nifedipine	+++	+	0
Amlodipine			
Nicardipine			
Isradipine			
Nitrendipine			
Feldodipine			
Nisoldipine	++++	0	0

+, + +, + + + — Degree of effect; 0, no effect.

Table 8
Alpha-subunit composition of voltage-gated calcium channels

Channel type	Alpha-subunit	Localization
High voltage activated		
L	1_C	Cardiac, smooth muscle[a]
L	1_D	Neuroendocrine
L	1_S	Skeletal muscle
P/Q	1_A	Neuronal
N	1_B	Neuronal
R	1_E	Neuronal
Low voltage activated		
T	1_G	Widespread
T	1_H	Widespread

[a]At least three splice variants exist-$1_{C\text{-}a}$ (heart); $1_{C\text{-}b}$ (smooth muscle, lung); $1_{C\text{-}c}$ (brain).

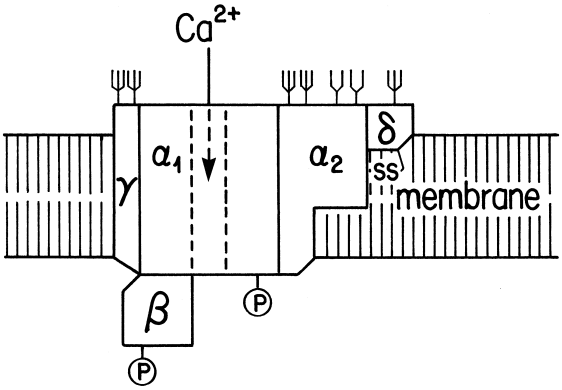

Fig. 10. A schematic representation of the organization of the *alpha-*, *beta* and *alpha*$_2$-delta subunits of the L-type voltage-gated Ca^{2+} channel. The *gamma* subunit is found only in the skeletal muscle variant of the L-type channel.

their therapeutic roles (Table 3). Consistent with their different pharmacological and therapeutic properties these agents also present different side effect and contraindication profiles (Tables 4 and 5).

When the second-generation 1,4-dihydropyridines are examined for their cardiovascular profile relative to verapamil, diltiazem and nifedipine it is clear that they are, like the first-generation 1,4-dihydropyridine, also vascular selective agents. It is also clear, however, they differ from nifedipine and amongst themselves in their degree of vascular:cardiac selectivity. These differences have been observed across a range of tissue preparations (Janis and Triggle, 1984; Perez-Viccaino et al., 1993; Godfraind, 1994), including human tissues (Schwinger et al., 1990; Table 6). These data suggest that there is a continuum of Ca^{2+} channel antagonist action in cardiac and vascular tissues (Table 7).

2.2. Mechanisms of selective actions of Ca^{2+} channel antagonists

It is clear that the Ca^{2+} antagonists are as a group, both first- and second-generation, not simply interchangeable agents. They exhibit significantly different pharmacological, therapeutic and contraindication profiles although they inhibit the same Ca^{2+} current through L-type channels. In principle, this selectivity of action derives from several sources including:

- Pharmacokinetic factors, including tissue distribution;
- Mode of calcium mobilization;
- Class and subclass of Ca^{2+} channel;
- State-dependent interactions;
- Pathologic state of tissue.

All of these factors contribute to the total selectivity profile of the available Ca^{2+} channel antagonists.

A principal contributor is that this class of drugs exhibits substantial selectivity for the L-type channel as opposed to the N, P, Q and R classes (Birnbaumer et al., 1994; De Waard et al., 1996; Walker and De Waard, 1998; Tables 8 and 9): this channel, although widely distributed is *functionally* of particular significance in the cardiovascular system. However, it is clear that discrete subtypes of these channels exist whose pharmacological specificity is determined by the structure of the major *alpha*$_1$-subunit

Table 9
The classification of voltage-gated calcium channels

Property	Class				
	T	L	N	P	R
Conductance, pS	7–10	11–25	10–20	10–20	15
Activation threshold	> -70 mV	> -30 mV	> -30 mV	> -30 mV	> -30 mV
Inactivation rate	fast	slow	intermediate	slow	fast
ms	~ 50	> 500	50–500	> 500	~ 30 m
Relative conductance	$Ba^{++} = Ca^{++}$	$Ba^{++} > Ca^{++}$	$Ba^{++} > Ca^{++}$	–	$Ba^{++} > Ca^{++}$
Open time duration ms	~ 1.0	~ 5.0	~ 1.0	4.0	
Pharmacological sensitivity:					
Dihydropyridines	–	×	–	–	–
w-CTX GVIA	–	–	×	–	–
w-CTX MVIIC	–	–	×	×	–
w-AGA IVA	–	–	–	×	–
w-AGA IIIA	–	×	×	×	–

and by the *beta*-subunit association (Birnbaumer et al., 1994; De Waard et al., 1996; Cai et al., 1997; Mitterdorfer et al., 1998; Walker and De Waard, 1998; Fig. 10). Unfortunately, comprehensive comparative pharmacology is not available for the L channel subtypes, but the available information clearly indicates that significant subtype discrimination by Ca^{2+} antagonists is probable (Welling et al., 1993, 1998; Hu and Marban, 1998). Recombinant cardiac and smooth muscle channels of the L class, $alpha_{1C}$a and $alpha_{1C}$b respectively, reveal differences in state-dependent interactions with 1,4-dihydropyridines (Welling et al., 1993, 1998).Although nisoldipine binds with the same affinity to these two splice variants, consistent with the identity of sequence in the binding regions of these two channels (IIIS5, IIIS6 and IVS6 segments), and interacts with the inactivated state of the channel in both cases, it also exhibits state-independent binding with the vascular smooth muscle channel. This is associated with the IS6 segment and accommodates a resting-state block of the vascular smooth muscle channel.

The state-dependent interactions of the Ca^{2+} channel antagonists have been well established through extensive electrophysiological investigations (Bean, 1984; Sanguinetti and Kass, 1984; Hondeghem and Katzung, 1985; Wibo, 1989; Nelson et al., 1990). Verapamil and diltiazem are prominently frequency-dependent and nifedipine and other 1,4-dihydropyridines show voltage-dependent interactions, whereby activity increases with maintained level of depolarization. These observations contribute, in significant part, to the observed anti-arrhythmic and cardiac depressant actions of verapamil and diltiazem and to the vasodilating properties of nifedipine and other 1,4-dihydropyridines. However, these electrophysiological investigations have not permitted a structure–function analysis of these state-dependent interactions. This analysis has been achieved through radioligand binding analysis of these drugs in intact cells under polarized and depolarized conditions (Wei et al., 1989; Zheng et al., 1992). From such studies it has been possible to examine the effect of structure in several series of 1,4-dihydropyridines on voltage-dependent binding and to show a correlation between the degree of such voltage-dependent binding and the vascular selectivity of these agents (Ferrante et al., 1989; Sun and Triggle, 1995). Radioligand binding for an activator–antagonist pair of 1,4-dihydropyridines revealed an absence of voltage-sensitivity for the activator S Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4(2-trifluoromethyl)phenyl-pyridine-5-carboxylate) and a significant voltage-dependence for the antagonist PN 200 110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-3-carbomethoxy-5-pyridinecarboxylate) (Table 10). Extension of these observations to three series of 1,4-dihydro-

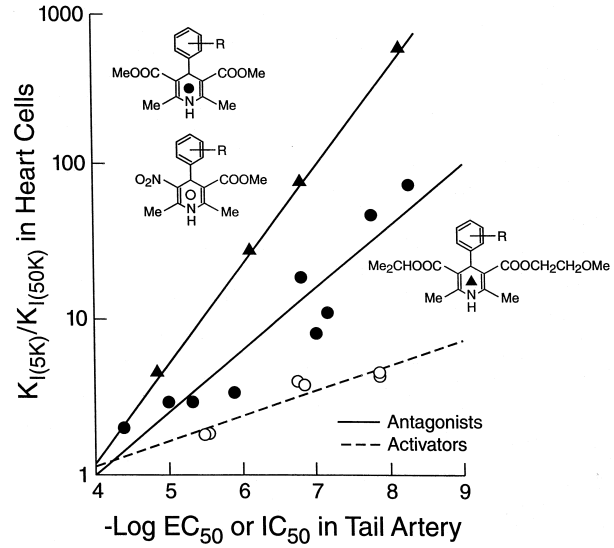

Fig. 11. The relationship between voltage-dependent binding of three series of 1,4-dihydropyridines in rat cardiomyocytes $K_I(5\ K)/K_I(50\ K)$ and antagonist (IC_{50}) or activator (EC_{50}) potency. Depicted is an antagonist series derived from nifedipine (0), an antagonist series derived from nimodipine ( ), and an activator series derived from Bay K 8644 ( ).

Table 10
1,4-Dihydropyridine binding to rat cardiomyocytes

K^+	[^{3}H]PN 200 110 (antagonist) K_D, $M \times 10^{-9}$	S [^{3}H] Bay K8644 (agonist) K_D, $M \times 10^{-9}$
5 mM	4.11	6.48
50 mM	0.07	2.86

Ferrante et al. (1989).

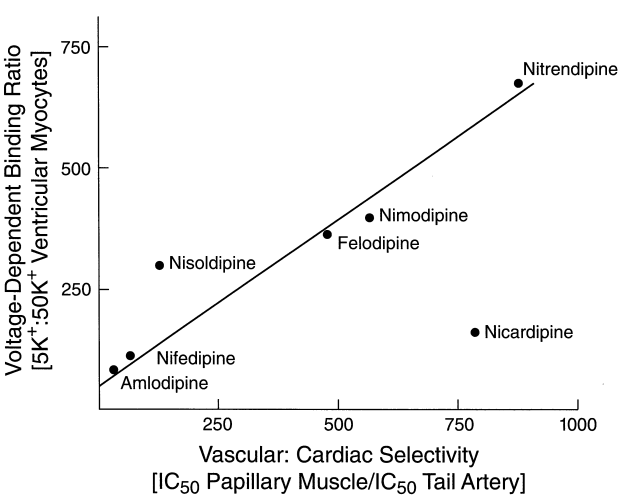

Fig. 12. The relationship between voltage-dependent binding and the vascular:cardiac selectivity of a series of clinically available 1,4-dihydropyridines. Voltage-dependent binding was determined in a radioligand binding assay in rat cardiomyocytes in the presence of 5 (polarized) or 50 mM K^+ (depolarized) and vascular:cardiac selectivity was determined in functional assays using rat tail artery and rat papillary muscle. Data from Sun and Triggle (1995).

pyridines structurally derived from the antagonists nifedipine and nimodipine and the activator Bay K 8644 revealed that both series of antagonists exhibited voltage-dependent binding to an extent determined by the substituents at C3 and C5 of the 1,4-dihydropyridine ring and that the degree of voltage-dependent interaction correlated well with antagonist potency. In contrast the activator series showed little voltage-dependent interaction irrespective of agonist potency (Fig. 11). Furthermore, for a series of clinically available 1,4-dihydropyridines there was an overall good correlation between the degree of voltage-dependent binding and experimentally measured vascular:cardiac selectivity (Sun and Triggle, 1995; Fig. 12).

2.3. Tissue selectivity and the cardiovascular safety of Ca^{2+} channel antagonists

Despite the prominence and the popularity of this class of drugs in cardiovascular disease, particularly in the control of hypertension they are not without their limitations. In particular, a number of recent studies have argued that the use of Ca^{2+} channel antagonists is associated with an increased risk of cardiovascular event (Pasty et al., 1995; Furberg et al., 1995; Katz, 1997; Epstein, 1998). Certainly, early trials with nifedipine, as the original rapid-acting formulation, had revealed its ineffectiveness or trend to harmful effects in post-infarct patients whilst verapamil,

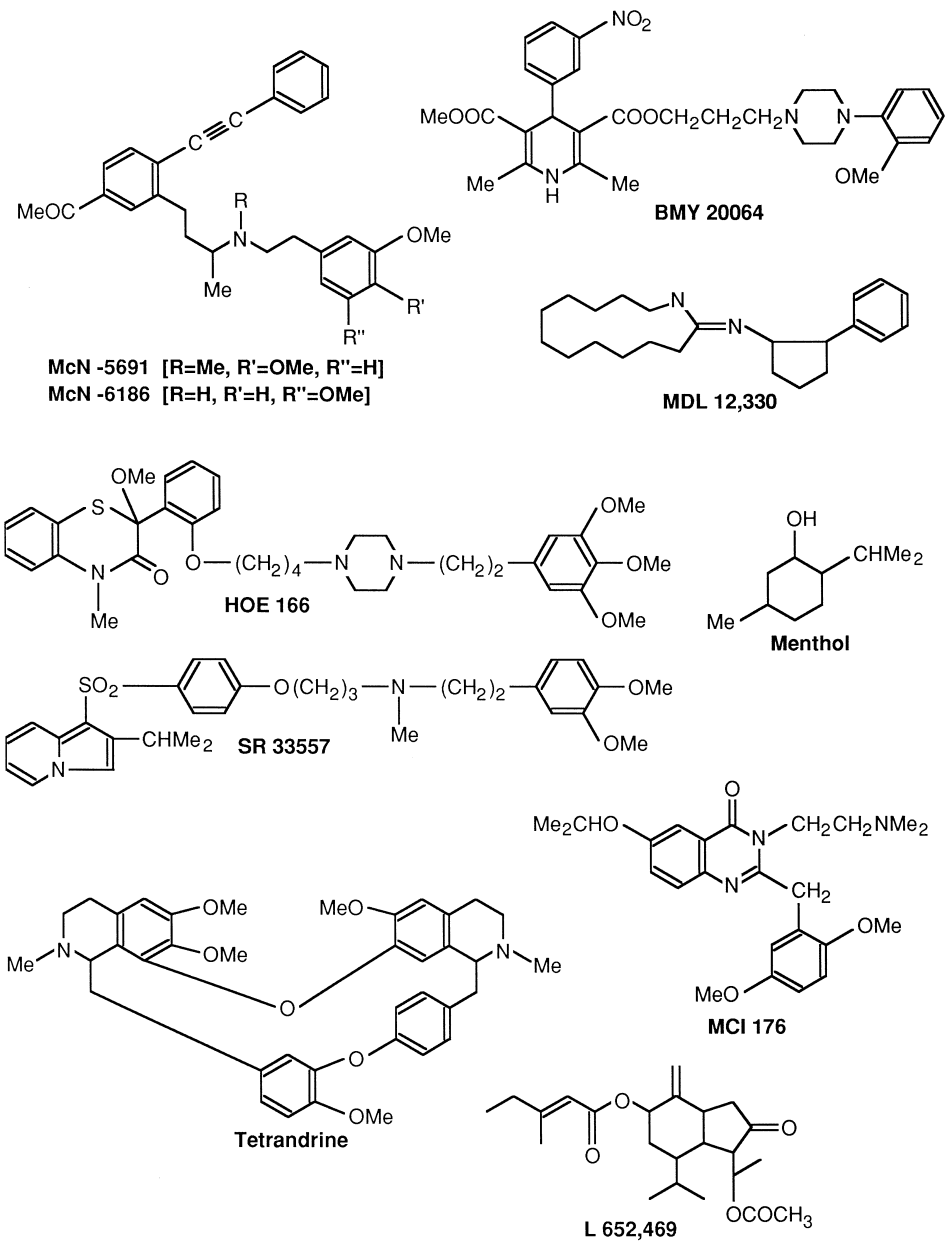

Fig. 13. Diverse chemical structures documented to have antagonist activity at L-type Ca^{2+} channels.

and to a lesser extent, diltiazem showed benefits notably in patients with good left ventricular function (Multicenter Diltiazem post-infarction trial research group, 1988; Danish Study Group on verapamil in myocardial infarction, 1990; Goldstein et al., 1991). Both pharmacokinetic and tissue selectivity factors seem likely to contribute to the deleterious effects of some Ca^{2+} channel antagonists, particularly in patients with compromised left ventricular function. As a consequence the FDA discouraged the use of short acting nifedipine in hypertension on the grounds that it failed to control blood pressure in a stable manner (Grossman et al., 1996; Marwick, 1996). Rapid acting vasodilators may be pro-ischemic and additionally will activate reflex sympathetic stimulation with attendant excess activation of the neurohormonal axis (Waters, 1991; Grossman and Messerli, 1997). These limitations do not appear to be apparent in long-acting agents, and the enhanced duration of action together with the increased vascular selectivity of the second-generation 1,4-dihydropyridines likely contributes to their safer profile (DEFIANT Research Group, 1992; Little et al., 1995; Abascal et al., 1998; Kloner et al., 1998).

3. Calcium channel antagonists: the future

The success in cardiovascular medicine of the existing Ca^{2+} channel antagonists owes much to their selectivity of action for specific subclasses of the L-type channel and the functional dominance of these subtypes in cardiac and vascular muscle physiology. Their success has, of course, raised substantial expectations that similarly specific antagonists of other classes of channel might also enjoy similar therapeutic success. These expectations have thus far not been realized, although considerable effort has been expended.

The neuronal localization of the N, P, Q and R types of high-voltage activated channel and their established role in the control of neurotransmitter release suggests potentially broad roles for selective antagonists or agonists in CNS (central nervous system)areas from affective disorders and analgesia through cognition and depression to neuroprotection and seizure control (Goldin et al., 1995; Little, 1995; Miljanich and Ramachandran, 1995; De Vry et al., 1997; Stefani et al., 1997; Helmeste and Tang, 1998). These channels are well characterized by toxins of the conotoxin and agatoxin families derived from molluscs and spiders respectively, and which have proved to be valuable pharmacological tools (Olivera et al., 1994; Olivera, 1997; Uchitel, 1997). Their multiple disulfide-bridge structure provides a rigid template around which selective amino acid change provides channel subtype selectivity. As peptides these agents provide the usual challenges to drug delivery and only one peptide, conotoxin MVIIA (SNX-111) an N-selective agent, has entered therapeutic trials for the control of pain and as a neuroprotective agent (Bowersox et al., 1996; Brose et al., 1997). These are likely to be, in any event, of limited application.

The challenge to the development of synthetic small organic ligands active as therapeutic agents at neuronal Ca^{2+} channels is actually two-fold. The first challenge is simply that of developing the appropriate screens and chemical libraries with which to search for lead structures. Given the very large number of diverse chemical structures that show activity at the L-type channel, in addition to the clinically available 1,4-dihydropyridine, phenylalkylamine and benzothiazepine structures that are clinically available (Rampe and Triggle, 1993; Fig. 13), it could well be

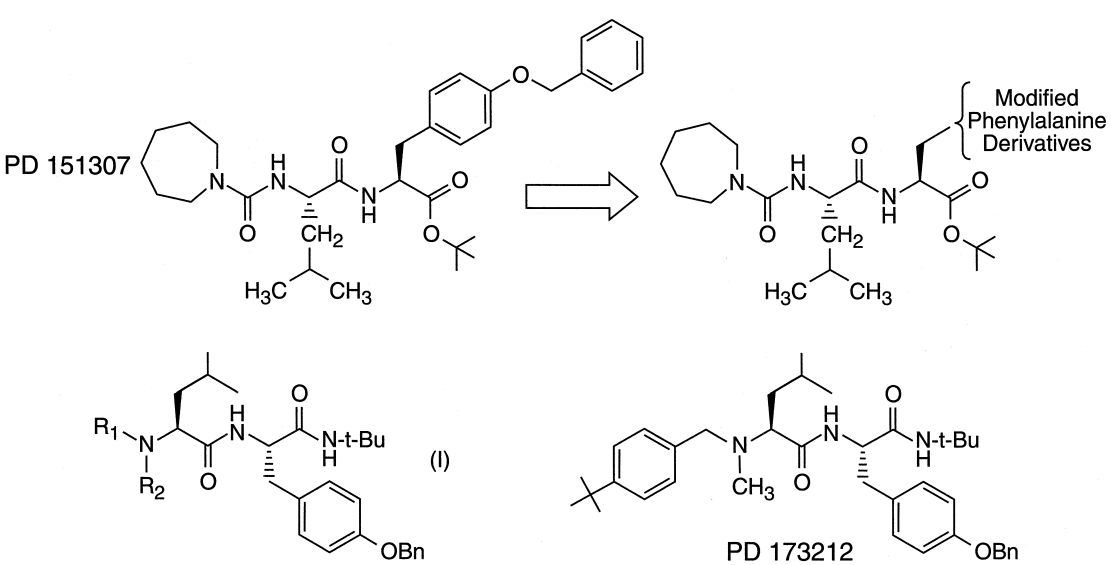

Fig. 14. Structures of new N-type Ca^{2+} channel active ligands (Chatterjee et al., 1999; Hu et al., 1999).

anticipated that a similar variety of structures should also be selectively active at the homologous N, P, Q and R channel types. However, subtype-selective leads have not been significantly reported and most of the molecules described are of both low potency and selectivity. Amongst those agents recently reported are fluspirilene at N-type channels (Grantham et al., 1994), the non-competitive NMDA receptor antagonists ifenprodil and eliprodil at N- and P-type channels (Biton et al., 1995; Bath et al., 1996; Church et al., 1997), the capsaicin receptor antagonist capsazepine acting non-selectively at neuronal channels (Docherty et al., 1997) and the aminoglycoside antibiotics at P/Q type channels (Pichler et al., 1996). Most recently, however, new compounds have been reported by Parke-Davis as N-type channel blockers. In a series of peptidylamines PD 151307 served as a lead for the development of the potent PD 173212 with activity at N-type channels in the nanomolar range (Chatterjee et al., 1999; Hu et al., 1999; Fig. 14). It is of interest that 1,4-dihydropyridines have also shown activity at N-type channels: typically, this activity is less than that exhibited at L-type channels, but these agents have been previously selected for their L-type activity in the cardiovascular system (Furukawa et al., 1997; Li et al., 1997, 1999; Uneyama et al., 1997). This suggests that a search of the 1,4-dihydropyridine structure might be a useful starting point for new N-channel structural leads. Encouragement for this is provided by the increasing number of observations that the 1,4-dihydropyridine structure may be a ''*privileged pharmacophore*'' capable of being appropriately ''*decorated*'' to provide a number of receptor-selective ligands and drugs (Fig. 15).

Table 11
Neuronal distribution of calcium channels

Neuron	% Current inhibition			
	Aga IVA	CTX GVIA	Nimodipine	Remaining
Purkinje	92	5	5	0
Hippocampal CA_1	26	37	19	Some
Hippocampal CA_3	14	21	36	25
Visual cortex	32	32	23	Some
Spinal cord	45	34	18	Some
Rat DRG	23	43	18	18
Rat sympathetic	0	93	7	2

Data from Mintz et al. (1992).

The second challenge to the discovery and therapeutic application of neuron-selective Ca^{2+} channel drugs is that of the regional and cellular heterogeneity of distribution of these channels. It is increasingly clear that neurons may and do express several channel types simultaneously, that

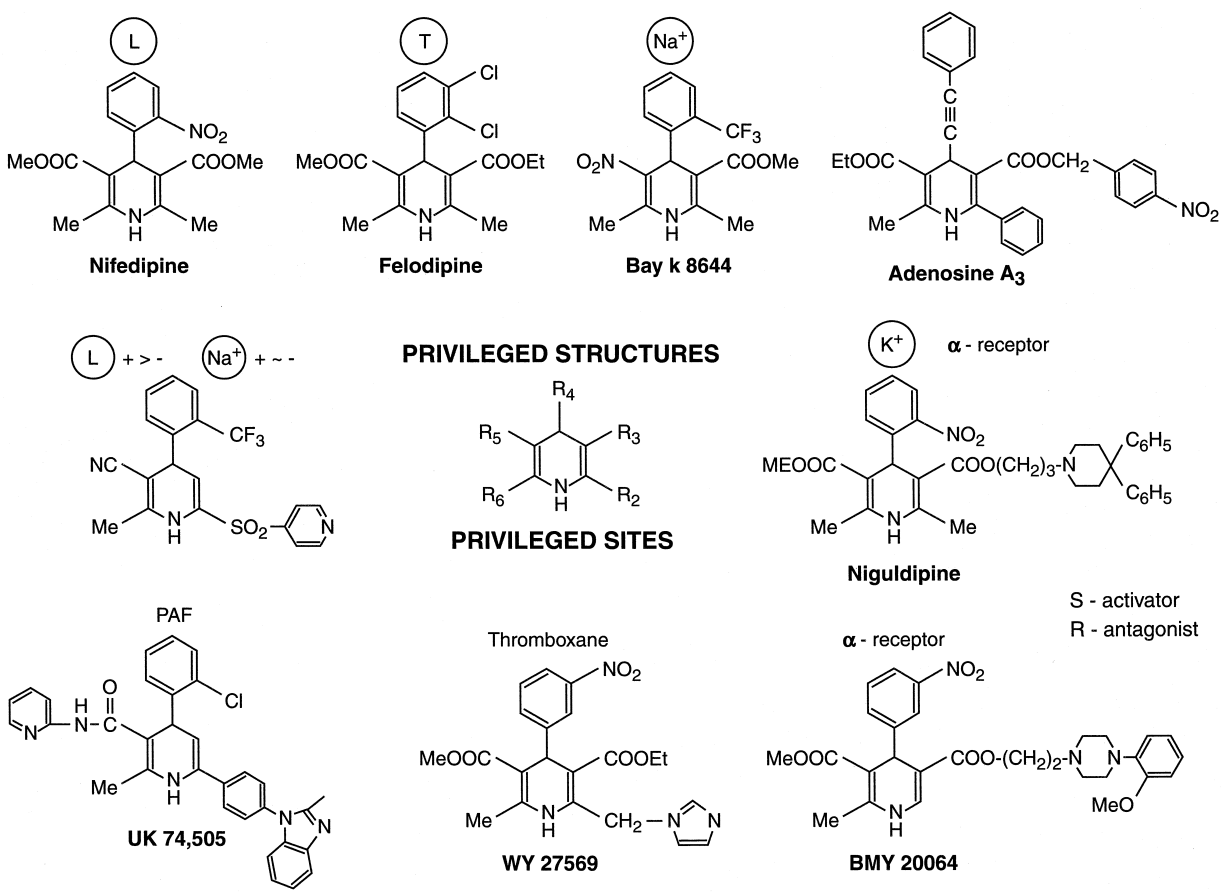

Fig. 15. The 1,4-dihydropyridine nucleus as a ''privileged structure'' or template for the design of receptor-active ligands.

the relative importance of expression of channel class may vary widely according to neuron type and localization (Table 11), and that there exists differential localization and function of channel types within a single neuron (Mintz et al., 1992; Westenbroek et al., 1992; Olivera et al., 1994; Wisgirda and Dryer, 1994; Cardenas et al., 1997). This heterogeneity will render the discovery of neuronal-selective Ca^{2+} channel therapeutic agents significantly more difficult than the corresponding discovery of the L-type Ca^{2+} channel antagonists.

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