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Commentary

Calcium channel antagonists: Clinical uses—Past, present and future

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ABSTRACT

The calcium channel antagonists are a mature group of drugs directed at cardiovascular diseases including hypertension, angina, peripheral vascular disorders and some arrhythmic conditions. Their sites and mechanisms of actions have been well explored over the past two decades and their interactions at the α_1 subunit of L-type channels ($Ca_V1.1$–1.4) have made them valuable molecular tools for channel classification and localization. With the realization that other members of the voltage-gated calcium channel family exist – $Ca_V2.1$–2.3 and $Ca_V3.1$–3.3 – considerable effort has been directed to drug discovery at these channel types where therapeutic prospects exist for a variety of disorders including pain, epilepsy, affective disorders, neurodegenerative disorders, etc. In contrast to the situation with the L-type channel antagonists success in developing small molecule antagonists of therapeutic utility for these other channel types has thus far been lacking. The reasons for this are explored and potential new directions are indicated including male fertility, bone growth, immune disorders, cancer and schistosomiasis.

1. Calcium channel antagonists: past

The term ''calcium channel antagonists'' refers to a chemically, pharmacologically and therapeutically heterogeneous group of drugs prominent both as cardiovascular therapeutic agents and as molecular tools. The prototypical agents of this group are diltiazem (a benzothiazepinone), nifedipine (a 1,4-dihydropyridine) and verapamil (a phenylalkylamine). The cardiovascular activities of these drugs as antihypertensive, antianginal and selective antiarrhythmic agents are due to their interaction at one particular calcium mobilization process—calcium entry through a voltage-gated calcium channel of the L-type. Many studies have demonstrated that in accord with their chemical heterogeneity these agents interact at discrete receptor sites associated with a major subunit of the channel (Fig. 1). The properties of these drugs and the mechanisms by which they function have been extensively reviewed during the past 25 years [1–4].

Controversy remains as to the credit for the discovery of this class of agents, although it is clear that they were not designed as drugs that would block calcium channel function [5]. Principal credit should go to Albrecht Fleckenstein at the University of Freiburg whose work demonstrated that verapamil and prenylamine produced effective electromechanical uncoupling in the heart, that these effects mimicked those of calcium removal, and that this uncoupling could be overcome by increasing extracellular calcium concentrations [1]. Almost simultaneously, Godfraind and Polster in Belgium noted the effects of cinnarizine and flunarizine on excitation–contraction coupling in vascular smooth muscle and wrote,

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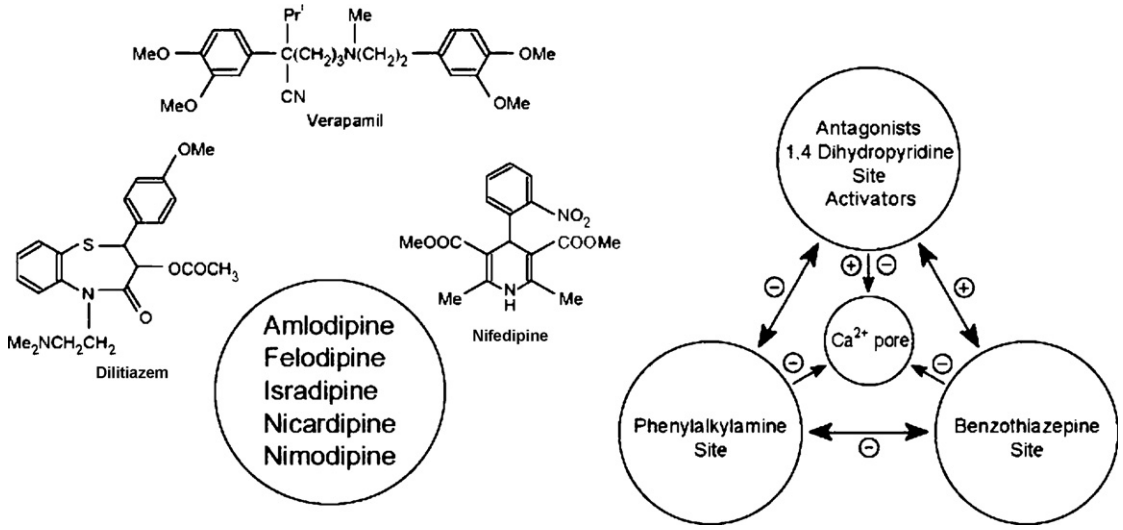
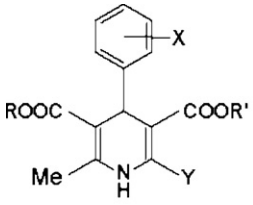


Fig. 1 – Ca^{2+} channel antagonist interactions at the L-type voltage-gated calcium channel. In this schematized representation the three major structural classes of drug are shown interacting at separate but allosterically linked receptor sites. The molecules depicted in the circle are second-generation 1,4-dihydropyridines and include the widely prescribed amlodipine (Norvasc™).

''la cinnarizine est un antagonist du calcium, au niveau de muscle vasculaire depolarise'' [6].

Following the introduction of these agents extensive work on second-generation drugs ensued. This led to the selective introduction of a number of agents of the 1,4-dihydropyridine class including amlodipine, benidipine, cilnidipine, felodipine, isradipine, lacidipine, lercanidipine, manidipine, nicardipine, nivadipine, nimodipine, nisoldipine, and nitrendipine ([3,4]; Fig. 2). Despite their close structural similarity to nifedipine and apparent ''me too'' status, these agents do differ in a number of important pharmacodynamic and pharmacokinetic properties, some of which are therapeutically significant, that are discussed in Section 3.

In the mid-1990s several cautionary notes were sounded concerning the safety of these agents (reviewed in [7]). These concerns included excess cardiovascular mortality [8], and increased risk of gastrointestinal bleeding and cancer [9,10]. A meta-analysis of trials of first-line antihypertensive agents indicated that hypertensive patients who had received calcium channel antagonists had a significantly increased risk of myocardial infarction relative to patients who had received β-blockers. Similarly, a cohort of elderly patients



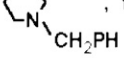
Amlodipine	X=2Cl; R=Me; R'=Et; Y=$CH_2OCH_2CH_2NH_2$
Benidipine	X=3NO_2; R=Me; R'=  ; Y=Me
Cilnidipine	X=3NO_2; R=$MeOCH_2CH_2$; R'=$CH_2CH{=}CH{\bullet}C_6H_5$(E); Y
Felodipine	X=2,3Cl_2; R=Me; R'=Et; Y=Me
Isradipine	X=  ; R=Me; R'=$CHMe_2$; Y=Me
Lacidipine	X=CH=CHCOOBu^t; R=Et; R'=Et; Y=Me
Lercanidipine	X=3NO_2; R=Me; R'=$CMe_2CH_2NMeCH_2CH_2CHPh_2$; Y=
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

Fig. 2 – 1,4-Dihydropyridine calcium channel antagonists.

taking calcium channel antagonists for hypertension had an increased risk of cancer development and the same authors proposed that these agents also increased the risk of gastrointestinal hemorrhage in hypertensive patients and, most surprisingly, that this risk was greater than for patients taking aspirin. These studies, essentially observational in character, have been discussed and criticized extensively (*inter alia* [7,11]). They suffer from the limitation common to all epidemiological studies, ''the difficulty in choosing groups of people who are alike in every way except for the exposure in question (cohort studies) or the disease in question (case control studies). Yet this is essential. Otherwise some difference between the groups might account for the results and badly mislead everyone'' [12].

However, despite significant and well-founded criticism of these studies important issues are raised. Thus, rapid-acting formulations of nifedipine can significantly increase cardiac rate through sympathetic reflex activation and may generate ''coronary steal''. This is a defect not associated with long-acting formulations or with second-generation intrinsically long-acting agents such as amlodipine. A second cautionary note dealt not with the mechanistic basis of any adverse effects of these agents, but rather with financial conflicts of interest. A survey of the literature for articles discussing the safety controversy between March 1995 and September 1996 revealed that authors who supported the safety of calcium channel antagonists were more likely than neutral or critical authors to have financial relationships with the manufacturers of these agents (96%, versus 60% and 37% respectively $P < 0.001$) [13]. This finding may, of course, reflect a genuine conflict, or may simply represent that authors with links to the industry were more knowledgeable, or had greater basic and clinical experience.

2. Calcium channel antagonists: present—clinical status

The calcium channel antagonists are one of seven principal classes of antihypertensive agents – the others being α-blockers, β-blockers, ACE inhibitors, AII antagonists, aldosterone antagonists and diuretics – each of which, save for aldosterone antagonists, has many members including both generic and non-generic agents. Therapeutic and economic questions are thus raised as to the appropriate choice of agent for the treatment of both simple and complex forms of hypertension [14,15]. Clinical trials are not simple to translate into the routine care of an essentially ambulatory and often silent disease such as hypertension that has multiple contributing causes, including lifestyle.

Resnick has argued that, ''You can't fool mother nature: different hypertensive people are different – the heterogeneity of hypertension'' [14]. Furthermore the various clinical trials including ALLHAT, the largest clinical trial for hypertension ever mounted, ANBP2 and ASCOT all have differences in study designs, risk profiles and baseline blood pressures. JNCVII (the Seventh Report of the Joint National Committee for the prevention, detection, evaluation and treatment of high blood pressure) continues to support the use of thiazide diuretics as a first step combined with drugs from other classes according to risk conditions [15]. Thiazide diuretics remain the best-evaluated agents and there is no disputing their beneficial effects in the reduction of hypertension-linked morbidity and mortality. However, the use of calcium channel antagonists is supported in the case of hypertension with typical complicating risks as coronary artery disease or diabetes. ALLHAT concluded that overall thiazide diuretics were as effective as the newer agents, but for those patients with diabetes amlodipine was as effective as chlorthalidone in all end points save heart failure [16]. However, in the Appropriate Blood Pressure Control in Diabetes trial (ABCD), a significantly smaller trial than ALLHAT, nisoldipine was inferior to lisinopril in reducing ischemic events and the trial was stopped prematurely [17]. Pharmacokinetic and pharmacodynamic differences between nisoldipine and amlodipine may account for this difference. In contrast, Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) that also compared newer antihypertensive drugs with older ones with endpoints of non-fatal myocardial infarction and fatal coronary heart disease, observed that amlodipine (with added perindopril as required) was, despite the premature termination of the trial, superior to atenolol (with added thiazide as required) [18]. Finally, Comparison of Amlodipine versus Enalapril to Limit Occurrences of Thrombosis (CAMELOT) compared amlodipine or enalapril with placebo in patients with coronary artery disease and normal blood pressure [19]. A reduction in clinical events (general incidence of cardiovascular events) was observed with amlodipine, but not with enalapril, although this conclusion should be tempered by a number of limitations of the study.

Calcium channel antagonists do continue to have a significant clinical role in the treatment of hypertension, particularly hypertension associated with complicating disorders of diabetes, coronary artery disease and where other agents are contraindicated. The current world market share of calcium channel blockers is approximately $6 billion and is dominated by amlodipine (Norvasc™), a share that seems likely to be maintained by newer combinations such as amlodipine plus Lipitor. However, given the number of drug classes and individual drugs, many of which are generic or will become generic in the next five years it is unlikely that there is a major market for new members of the existing class of calcium channel antagonists in cardiovascular disease.

3. Calcium channel antagonists: present—mechanistic status

Despite the widespread distribution of voltage-gated calcium channels in the cardiovascular system, neuronal tissues and secretory cells the existing antagonists do exhibit substantial cardiovascular selectivity. The basis for this selectivity of action has been investigated extensively, and offers valuable lessons for drug discovery and design. In principle, this selectivity arises from several factors, alone and/or in combination:

1. Mode of calcium mobilization—what source of calcium is activated.
2. Class and subclass of channel.

3. Pharmacokinetic factors.
4. State-dependent interactions.
5. Tissue and cellular pathology.

The two most critical factors are the class and subclass of channel and the prominent state-dependent interactions exhibited by existing calcium channel antagonists, particularly those of the 1,4-dihydropyridine class.

The L-type channels, sensitive to diltiazem, nifedipine and verapamil, are of the Ca_V1 class (Table 1). The major pore-forming α_1 subunits depicted there comprise the high voltage-activated channels, $Ca_V1.1$–1.4 (L-type) and $Ca_V2.1$–2.3(P/Q-, N- and R-type, respectively) and the low voltage-activated channels $Ca_V3.1$–3.3 (T-type). Associated with this major subunit are $\alpha_2\delta1$–4 subunits, $\beta1$–4, and $\gamma1$–8 subunits. These subunits have significant modulating influence on the expression, biophysical and pharmacological properties of the α-subunit (for reviews see [4,20]). Additional complexity is provided by the extensive number of splice variants of the α_1 subunits and the widespread and heterogeneous cellular distribution of the various channel types and isoforms [20].

Drug binding sites exist on the α_1 subunit, with exceptions for gabapentin and pregabalin which interact with an $\alpha_2\delta$ subunit, and these have been particularly well characterized for diltiazem, nifedipine and verapamil including the identification of specific amino acid residues critical for high affinity interaction. These residues on domains III and IV of the α_1 subunit are allosterically linked to the channel pore and gating machinery [21]. These antagonists, and the second-generation 1,4-dihydropyridines, appear to have a considerable selectivity for the L-type channel in the cardiovascular system thus accounting for their general inactivity in neuronal and secretory tissues. Additionally, different splice variants of $Ca_V1.2$ are expressed in cardiac and vascular tissue and the latter variants appear to have a higher affinity for 1,4-dihydropyridines [22]. This differential pharmacological sensitivity is coupled with a high and structure-dependent voltage-sensitivity of 1,4-dihydropyridine interactions whereby affinity is significantly enhanced in the depolarized state [23]. These two features contribute to the higher vascular selectivity (including regional vascular bed selectivity) seen with 1,4-dihydropyridines. A linear relationship exists between voltage-dependent binding and vascular selectivity for clinically available 1,4-dihydropyridines whereby vascular selectivity increases with increasing voltage-dependence of binding [24].

Table 1 – Voltage-gated calcium channels: their classification and pharmacology

	L	T	N	P	Q	R
Types of current						
Calcium channels	$Ca_V1.1$–1.4	$Ca_V3.1$–3.3	Cav2.1–2.3	Cav2.1–2.3	Cav2.1–2.3	Cav2.1–2.3
Activation threshold	High	Low	High	High	High	High
Function	E–C coupling CV system, smooth muscle, gene expression endocrine cells, neurotransmitter release	Cardiac sinoatrial node spiking, repetitive activity in neurons and endocrine cells, smooth muscle	Neuronal only, neurotransmitter release	Neuronal only, neurotransmitter release	Neuronal, neurotransmitter release	Neuronal, neurotransmitter release
Pharmacology[b]						
1,4-Dihydropyridines	Sensitive	Insensitive	Insensitive	Insensitive	Insensitive	Insensitive
Verapamil	Sensitive	Insensitive	Insensitive	Insensitive	–	Insensitive
Diltiazem	Sensitive	Insensitive	Insensitive	Insensitive	–	Insensitive
Mibefradil	Insensitive	Sensitive	–	–	–	–
ω-Conotoxin GVIA (C 9915)	Insensitive	Insensitive	Sensitive	Insensitive	Insensitive	Insensitive
ω-Conotoxin MVIIC (C 4188)	Insensitive	Insensitive	Sensitive	Sensitive	Sensitive	Insensitive
ω-Agatoxin IVA (A 6719)	Insensitive	Insensitive	Insensitive	Sensitive	Sensitive	Insensitive
ω-Agatoxin IIIA	Sensitive	Insensitive	Sensitive	Insensitive	Sensitive	Sensitive
Tissue expression	Widespread: CV system, neurons, endocrine, skeletal muscle	Neurons, smooth muscle, sinoatrial node	Neurons	Neurons	Neurons	Neurons
Disease relevance	Cardiovascular disorders	Arrhythmias, epilepsy, fertility?	Pain	Migraine? Epilepsy	Migraine? Epilepsy	Diabetes?

4. Calcium channel antagonists: future

Although the cardiovascular properties of the calcium channel antagonists have dominated their clinical applications for the past thirty years there has been considerable ongoing activity in the search for and the application of drugs active at non-L-type calcium channels. Clinical success with selective blockers of these channels has, thus far however, been quite limited and restricted to one peptide and two small (and structurally related) molecular species [25–28]. Since the N-, P/Q- and R-type channels are associated exclusively with the peripheral and central nervous systems and T-type channels with both

cardiovascular and neuronal tissues considerable potential scope is believed to exist to duplicate the clinical success of the L-type channel antagonists with particular applications to epilepsy and pain where there exists substantial unmet medical need. However, an examination of the general roles and distribution of voltage-gated calcium channels suggests potential clinical roles in disease states from achalasia to vertigo [2,5]. Success in any of these clinical situations will depend on several factors alone or in combination including: (a) unique localization of a channel type or subtype within a particular cell or tissue type; (b) specific functional activation in a particular cell/tissue type or disease state; (c) specific regulation of a channel type- or subtype in a disease state; (d) the ability to deliver drug specifically to a particular cell or organ type. The success of the existing calcium channel antagonists in cardiovascular disease hinges largely upon factor b.

Proof of principle for application in pain has been obtained with the clinical success of the peptide toxin ziconotide in chronic neuropathic pain acting through the N-type channel and similarly the widespread utility of the small molecule gabapentin that acts through an $\alpha_2\delta$ subunit of the channel. These areas have been extensively reviewed recently [27,28] as has the potential role of calcium channel antagonists in epilepsy [29,30]. Hence, attention will be directed to some newly emerging areas of potential therapeutic utility including fertility control, bone remodeling, immune function, cancer and parasite control.

4.1. *Control of fertility: calcium channel antagonists as contraceptive agents*

The interaction of egg and sperm is organized around several Ca^{2+}-dependent processes of capacitation, including the activation and hyperactivation (motility), and the acrosomal (exocytotic fusion) phases [31,32]. Agents targeting calcium channels might thus have a role as male contraceptive agents. A multiplicity of high- and low-voltage calcium channels have been functionally and biochemically detected in mammalian sperm, but their individual roles in the calcium mobilization responses and fertilization response remain to be completely elucidated [33,34]. Of particular interest is the report of $Ca_V2.2$ (N-type) and $Ca_V2.3$ (R-type) channels in mouse sperm [35]; these channel classes have been customarily assigned to a solely neuronal localization. Epidemiological reports exist for male patients taking calcium channel antagonists for hypertension whose fertility was restored upon cessation of therapy [31,36]. The significance of these findings remains to be determined. It is, however, most improbable that any pharmaceutical company will undertake clinical trials as a contraceptive agent of a calcium channel antagonist already marketed for cardiovascular disease. However, if such a role was established the off-label use might well be considerable!

Targeted gene disruption studies indicate, however, that the Ca_V1–Ca_V3 classes are not essential for male fertility, but particular attention is drawn to the more recently discovered CatSper class of channels that are specifically localized to sperm and spermatogenic cells [37–41]. Four members of this class exist – CatSper1–4 – and their sequence resembles that of a single domain of the voltage-gated calcium channel α_1 subunit. Since they are co-expressed it is possible that CatSper1 and 2 form homo- or heteromeric calcium-selective channels uniquely associated with flagella function. Three lines of evidence in particular point to a particular role for CatSper channels in sperm function. First, CatSper1$^{-/-}$ mice have sperm with decreased motility that are unable to fertilize intact eggs and CatSper2$^{-/-}$ mice are completely infertile [37,39], second, CatSper expression in mice is linked to the development of sexual maturity. The third line of evidence is that CatSper gene expression is reduced in subfertile males with sperm of reduced motility [42,43].

There are no published data on the pharmacology of the CatSper class of channels: one suspects, however, that proprietary research is ongoing. An obvious lead structure for investigation would the 1,4-dihydropyrdine nucleus since, properly decorated, this is a remarkably promiscuous ligand for ion channels [44]. However, the search for male contraceptive drugs is a difficult one subject as it is to ''the tyranny of numbers''—one sperm is all that is necessary!

4.2. *Bone remodeling: a role for calcium channel activators?*

The remodeling of bone depends on the continuous interaction between osteoblasts, the bone-forming cells, and osteoclasts, the bone resorbing cells. Vitamin D and parathyroid hormone and mechanical strain increase bone formation and a role for L-type Ca^{2+} channels is well established [45]. Application of strain to osteoblasts in the presence of the L-type 1,4-dihydropyridine Ca^{2+} channel activator Bay K 8644 increases both Ca^{2+} uptake and the levels of the extracellular matrix proteins [46].

The 1,4-dihydropyridine Ca^{2+} channel activators have been extremely useful pharmacological and biophysical tools, but have found no therapeutic applications largely because their actions are widespread and involve a general activation of L-type Ca^{2+} channels. The application of Bay K 8644 encapsulated in a poly-L-lactic acid (PLLA) scaffold is of obvious interest since it may permit the local stimulation of bone growth without the simultaneous (and detrimental) activation of L-type Ca^{2+} channels that are widespread in cardiovascular and neuronal tissues [47,48].

4.3. *Cancer chemotherapy*

Observational studies discussed earlier suggested a link between calcium channel antagonist use and increased risk of cancer. Although not confirmed there is a plausibility behind the proposed link since Ca^{2+} has long been recognized as a critical cellular intermediate in cellular growth, differentiation, proliferation and cell death and in model studies existing calcium channel blockers have demonstrated an ability to inhibit tumor cell growth (*inter alia* [49]) (for recent reviews of the roles of ion channels, including calcium channels, in cancer see [50,51]). In fact, in one clinical study an inverse association was noted between prostate cancer and verapamil use [52].

More focused attention has been directed recently at T-type voltage-gated calcium channels and the possible role of T channel selective drugs for several reasons. T-type channels are well recognized to be linked to differentiation and

proliferation processes, to be re-expressed in a number of malignant states and to be involved in calcium-mediated cell death (for the most recent review see [53]). T-type calcium channels may be specifically appropriate to these events because of their ability, as low voltage-activated channels, to generate low threshold calcium spikes and to generate ''window'' calcium currents at resting membrane potentials. Some evidence suggests that the $Ca_V3.1$ and $Ca_V3.2$ channels may be particularly important in cellular proliferation and differentiation and growth, respectively.

Although not a specific T-type antagonist mibefradil has demonstrated anti-proliferative properties [54]. More recently, two series of compounds with reported T-type selective properties have been reported to have anti-proliferative actions in tumor cells at micromolar concentrations [55,56]. A series of compounds with structural resemblance to flunarizine and cinnarizine has suggested a pharmacophoric structure that includes two aromatic regions linked by a amine containing straight chain [55]. The other series consists of 3,4-dihydroquinazolines also active at micromolar concentrations [56]. However, no data are available to indicate the selectivity of these series for T-type channels over other voltage-gated calcium channels or over other sites of calcium interaction, including calmodulin. Given the ability of diverse compounds, including neuroleptics [57], to interact with T-type calcium channels evidence for selectivity of action is particularly important.

Before a role for T-type channel antagonists can be pursued further it will be important to have more selective compounds and to know the effects on phenotype of T channel knockouts.

4.4. *Schistosomiasis (and tropical disease targets?)*

Schistsomiasis (also known as bilharziasis) ranks second behind malaria in its public health and economic importance in tropical and subtropical areas of the world. Villages such as Kwa'al in Nigeria are referred to as ''schisto'' villages because over one-third of the children have blood in their urine from infection by this parasitic disease [58]. The anthelmintic agent praziquantel remains the agent of choice for the treatment of schistosomiasis where it functions by interfering with calcium homeostasis, by mechanism(s) unknown until recently [59]. Recent work suggests that praziquantel interacts by activation of a voltage-gated calcium channel thus causing excessive influx of calcium into the parasite and ensuing paralysis and death [60]. The interaction is unusual since it involves a β-subunit unique to the schistosome flatworm whose interaction with the pore-forming α_1 subunit is inhibitory to channel function rather than stimulant to function and expression as seen with mammalian β-subunits. Expression of the schistosomal β-subunit with a mammalian $Ca_V2.3$ α_1 subunit results in a reduction in current amplitude and partial sensitivity to praziquantel at 100 nM [61,62]. Interaction of praziquantel with the schistosome β-subunit thus relieves a unique inhibitory α_1–β interaction. Partial sensitivity of praziquantel action to the 1,4-dihydropyridines nicardipine and nifedipine was observed and the inhibitory effect of cytochalasin D, a cytoskeleton depolymerizing agent, offer some additional support to this proposed mechanism of action of praziquantel [63].

The praziquantel story is of considerable interest for two reasons. First, it may define the mechanism of action of an important tropical disease drug and second, in common with the work defining the $\alpha_2\delta$-subunit as the site of action of gabapentin and pregabalin [64] it defines the α_1–β interface as a new target for drug discovery for voltage-gated calcium channels.

4.5. *Control of immune function: voltage-gated channels in lymphocyte cells*

Calcium plays critical roles in T cell and T helper cell activation following antigen arrival including both persistent and oscillatory increases in $Ca^{2+}{}_{int}$ that mediate proliferation, gene transcription, cytokine production, etc. The general assumption has been, since both T and B cells are non-excitable, that the rise in $Ca^{2+}{}_{int}$ has been mediated through calcium-release-activated-channels (CRAC) [65]. However, evidence has steadily accumulated for the biochemical and functional significance of voltage-gated calcium channels in lymphocyte function [66,67]. These lines of evidence include the detection of $Ca_V1.1$–1.4 channels in a number of human and murine T cells and T cell lines including several splice variants [68,69], and the ability of calcium activators and antagonists, principally of the 1,4-dihydropyridine class, to modulate T cell function.

However, these channels appear to be distinct from those that function in conventionally excitable cells. In Jurkat leukemia cells the major channel protein expressed was $Ca_V1.2$, but in truncated form and in human T lymphocytes the Cav1.4 protein variants expressed lacked the voltage sensor sequence of motif IV, an omission that likely determines the voltage-insensitivity to activation of these variants [68,69]. Additionally, the concentrations of calcium drugs employed in T cell studies have typically been significantly greater than those employed for classical L-type channels, thus raising questions of specificity of action. Overall, the available evidence suggests that T (and B) lymphocytes do express unique proteins of the Ca_V1 class, that these are involved in mediating physiologically and pathologically important calcium fluxes, but that these lack voltage-dependence of action, are not activated by membrane depolarization, and exhibit low affinity pharmacology to the available L-type antagonists and activators of the 1,4-dihydropyridine class. A continued search for new ligands of higher affinity would thus appear to be important.

The 1,4-dihydropyridine privileged structure will be a useful starting point in this search for new ligands. At least two additional studies support this view. T helper type 2 cells secrete a number of interleukins and are critically involved in the elimination of extracellular pathogens. These cells express functional 1,4-dihydropyridine receptors involved in calcium mobilization sensitive to activators and antagonists. Activation of these channels by mercury in Brown Norway rats generates IL-4 and autoimmune glomerulonephritis that is blocked by the 1,4-dihydropyridine antagonist nicardipine [70]. This calcium mobilization pathway is apparently mediated through a cGMP–protein kinase G pathway [71]. T lymphocytes express β3 and β4 subunits and Ca_V β-deficient T cells showing an impaired calcium response upon activation are impaired in cytokine production and nuclear factor activation [72]. This is consistent with earlier studies in

lethargic mice that have a generalized immune disorder resulting from a mutation in the Ca_V β4 subunit gene [73].

5. Calcium channel antagonists: conclusions

Without exception, the drugs available with therapeutic activity mediated through voltage-gated calcium channels are antagonists. Activators do exist, almost exclusively belonging to the 1,4-dihydropyridine class: these are useful as molecular tools but have achieved no therapeutic utility. Furthermore, with three exceptions, the available drugs are all antagonists at the L-type channel where they mediate their diverse cardiovascular effects. These exceptions are ziconotide, a peptide toxin interacting at N-type channels and used for the relief of chronic neuropathic pain [74], and the small molecule agents pregabalin and gabapentin, synthesized originally as GABA-mimetics, but known to exert their actions in pain through interaction at an $\alpha_2\delta$ subunit of the channel [64].

The ready availability of drugs interacting at L-type channels and the corresponding paucity at other calcium channel types despite potentially attractive therapeutic uses and considerable effort provides valuable lessons in the strategy of drug discovery and development.

1. Diltiazem, nifedipine and verapamil (and related molecules including prenylamine, bepridil, etc.) were discovered in the absence of any structural or much functional knowledge of voltage-gated calcium channels. Knowledge of channel structure or classification contributed nothing to the discovery process and these molecules were not synthesized as part of any ''calcium channel drug program''. This is not, of course, to argue that the subsequently obtained and detailed knowledge of calcium channel structure and function will not yield knowledge of value for drug development, but rather that such knowledge is not an obligatory prerequisite. Structural knowledge has proved useful in the determination of the sites of action of gabapentin and pregabalin at the $\alpha_2\delta_2$ subunit, but like the L-type channel drugs these agents were also discovered during a search for drugs acting at non-channel targets.
2. The biological assays used for the evaluation of the prototypical calcium channel antagonists, blood pressure measurements and *in vivo* and *in vitro* cardiac and vascular smooth muscle preparations, were closely related to the therapeutic endpoints targeted—angina and hypertension.
3. The L-type channel drugs exhibit significant state-dependent interactions interacting preferentially at open or inactivated states of the channel. This permits selective interaction at arrhythmic cardiac cells (verapamil) and vascular smooth muscle beds in tonic contraction (nifedipine). The general absence of sustained (tonic) depolarization in neurons, save in some pathological conditions, likely underlies the general absence of activity of these agents in such cells. Absent such state-dependent interaction a non-selective blockade of L-type channels would occur leading to a general circulatory collapse and significant neuronal and secretory cell inhibition. Such findings would likely have deterred further investigation.
4. The L-type calcium channel dominates the activity of the cardiovascular system; other calcium channel types contribute comparatively little to the mechanical activity of the cardiovascular system. In neuronal tissues a variety of calcium channel types contribute to neuronal activity and the relative distribution of channel types is both cell- and species-dependent. In marked contrast to the situation in smooth and cardiac muscle, neuronal disorders such as pain and ischemic brain damage involve multiple pathways, including multiple calcium channel pathways. The documented ineffectiveness of calcium channel antagonists in stroke has several likely origins including the activation of multiple Ca^{2+} channel mobilizing pathways during the ischemic insult [75]. Blockade of a single channel type is thus not an effective strategy, in marked contrast to the success of such strategy in the cardiovascular system. Effective strategies for ischemic stroke will have to be either ''cocktails'' of drugs interacting selectively at different sites, promiscuous ligands that can interact with multiple sites, or the targeting of a pathway or pathways, perhaps an anti-oxidant mechanism, that is common to all the pathways of insult.

Nonetheless, it is clear that the non-L-type voltage-gated calcium channels do offer significant opportunities for new therapeutic directions from achalasia to vertigo. Pain and epilepsy continue to be major areas of attention with promising new molecules becoming available. New structural information points to new sites for drug interaction including the $\alpha_2\delta_2$ subunit and the α_1–β subunit interface. Finally, the discovery of the role of voltage-gated calcium channels in sperm and the fertilization response and the reports of sperm specific calcium channels may offer interesting new directions for contraception and birth control.

Conflicts of interest

I have no research support from private sources. I give seminars at universities and pharmaceutical companies in North America and abroad for which I decline honoraria, but accept travel reimbursement.

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