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COMMENTARY

SITES OF ACTION OF Ca^{2+} CHANNEL INHIBITORS

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Fleckenstein [1] was the first to use the term "calcium antagonists" for drugs that interfere with the contractile effect of Ca^{2+} on cardiac and smooth muscle. Recently, various investigators attempted to find a more specific term for the same group of drugs [2–4]. The terms "Ca^{2+} blockers," "Ca^{2+} entry blockers," "Ca^{2+} channel blocking drugs" or "Ca^{2+} channel inhibitors" have been used. It has been proposed [5, 6] that the term "Ca^{2+} antagonists" be retained as a broader term for drugs whose major pharmacologic action is to prevent Ca^{2+} entry into the cell, lower cytosolic Ca^{2+} by other mechanisms, or inhibit the effect of Ca^{2+} by acting on Ca^{2+} binding proteins in cytosol. A subgroup of Ca^{2+} antagonists that inhibits Ca^{2+} channels in the cell membrane can then be called Ca^{2+} channel inhibitors. This subgroup includes, among other drugs, verapamil, the 1,4-dihydropyridines, and diltiazem. This review will adhere to the above described terminology.

The chemical structures for the first generation of Ca^{2+} channel inhibitors are shown in Fig. 1. The basis for therapeutic use of Ca^{2+} channel inhibitors in the treatment of hypertension and coronary and other vascular spasms is a relatively selective relaxation of vascular smooth muscle that is probably caused primarily by an inhibition of Ca^{2+} entry into vascular smooth muscle cells. At concentrations causing vasodilatation, some of these drugs, e.g. the 1,4-dihydropyridines, are relatively ineffective as myocardial depressants or as inhibitors of excitation–secretion coupling. The major site of action of Ca^{2+} channel inhibitors appears to be the cell membrane, but it is not certain whether these drugs interact with Ca^{2+} channels directly or modify other proteins that control Ca^{2+} channels. Intracellular sites of action have been proposed for some Ca^{2+} channel inhibitors [7–9]. It remains to be determined, however, whether these additional effects contribute to, or modify, the therapeutic profile of some of these drugs. This article will review the current state of knowledge on the sites of action for Ca^{2+} channel inhibitors.

EVIDENCE FOR SARCOLEMMAL SITES OF ACTION

Inhibition of contraction and $^{45}Ca^{2+}$ influx

Evidence that the major site of action of the Ca^{2+} channel inhibitors is not contractile or associated regulatory proteins is derived partly from experiments with skinned muscle fibers. Verapamil, diltiazem and nifedipine, even at 100 μM, were reported not to inhibit the Ca^{2+}-induced contractions of skinned smooth muscle preparations [10]. The 1,4-dihydropyridines (Fig. 2), nifedipine [10], nisoldipine [11], and nitrendipine [12], had only a slight inhibitory effect on Ca^{2+}-induced contractions of skinned smooth muscle and then only at concentrations 500–1000 times higher than required to antagonize Ca^{2+}-induced contractions of K^+-depolarized vascular smooth muscle. Verapamil even increased Ca^{2+}-induced contractions in skinned smooth muscle [13]. Skinned cardiac fibers were also found to be insensitive to verapamil [14].

Further evidence for an action at the sarcolemma is the inhibition of $^{45}Ca^{2+}$ influx into the vascular smooth muscle by Ca^{2+} channel inhibitors in a high K^+ or norepinephrine (NE)-containing medium. $^{45}Ca^{2+}$ uptake was found to be inhibited in rabbit aortic rings by gallopamil (D600, [15]), nitrendipine [16], nisoldipine [17], diltiazem [18] and flunarizine [19]. The inhibition of $^{45}Ca^{2+}$ uptake in rabbit aortic rings closely paralleled the inhibition of K^+-induced contractions. Contractile responses to NE at high concentrations were found to be considerably more resistant to Ca^{2+} channel inhibitors than NE-stimulated $^{45}Ca^{2+}$ influx [17, 18]. For some tissues, particularly large arteries and aorta, this last observation can probably be attributed to the finding that NE-induced contractions are mediated to a large extent by release of Ca^{2+} from intracellular storage sites, rather than by increased entry of extracellular Ca^{2+}.

Ca^{2+} channel inhibitors reduce preferentially the second phase of the vasoconstrictor response to norepinephrine or to electrical stimulation of isolated vessels [20–22]. The second sustained phase of the contractile response is not seen in a Ca^{2+}-free medium and is apparently mediated by exogenous Ca^{2+} [23]. The selective effect on the second phase of the response suggests that these drugs act at the cell membrane rather than on the intracellular translocation of Ca^{2+}. Also, Ca^{2+}-induced constriction of rabbit ear artery is effectively antagonized by Ca^{2+} channel inhibitors, e.g. nitrendipine [24].

Inhibition of Ca^{2+} current

Even more convincing evidence for a sarcolemmal site of action of Ca^{2+} channel inhibitors [25–27] comes from electrophysiological studies. The transmembrane inward current (I_{si}), which is carried mainly by calcium ions in cardiac tissue, contributes

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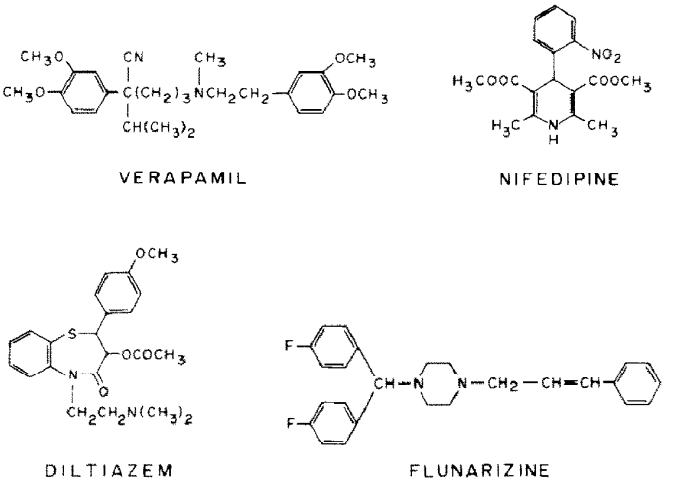
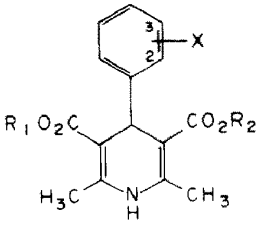


Fig. 1. Structural formulae of first generation Ca^{2+} channel inhibitors.

to the plateau phase of the action potential. An ion current that repolarizes the membrane is a delayed rectifier (I_x), which is carried largely by potassium ions. Nisoldipine was reported to selectively block I_{si} without reducing I_x, whereas gallopamil (D600) blocked both currents at similar concentrations [28]. The blockade of I_{si} in cardiac conducting tissue can be expected to reduce re-entrant rhythms and the initiation of spontaneous firing; this represents one of the mechanisms of antiarrhythmic action of Ca^{2+} channel inhibitors.

Recent electrophysiological studies have also clarified several other points related to the sites and mechanisms of action of these drugs. Lee and Tsien [26] have shown that verapamil and gallopamil, like the 1,4-dihydropyridines and the inorganic Ca^{2+} channel inhibitors, do not slow activation of Ca^{2+} channels, and that all of the organic agents, including nitrendipine, exhibit increased block with increased frequency of stimulation. Thus, the 1,4-dihydropyridines do not simply plug the channel from the outside in a manner analogous to tetrodotoxin block of Na^+ channels in nerve. Ca^{2+} channels undergo transitions between resting (closed but available for activation), open (Ca^{2+} conducting), and inactivated (closed but unavailable for activation) states. Lee and Tsien [26] have confirmed previous studies [29] indicating that verapamil and gallopamil block Ca^{2+} channels only after they are either in the opened or the inactivated state, and that the degree of block



DRUG	X	R_1	R_2
NITRENDIPINE	3-NO_2	CH_3	C_2H_5
NIMODIPINE	3-NO_2	$CH(CH_3)_2$	$(CH_2)_2OCH_3$
NIFEDIPINE	2-NO_2	CH_3	CH_3
NISOLDIPINE	2-NO_2	$CH_2CH(CH_3)_2$	CH_3
NILUDIPINE	3-NO_2	$(CH_2)_2OC_3H_7$	$(CH_2)_2OC_3H_7$
FELODIPINE	2,3-Cl	CH_3	C_2H_5
NICARDIPINE	3-NO_2	CH_3	$(CH_2)_2N(CH_3)CH_2C_6H_5$

Fig. 2. Structural formulae of some 1,4-dihydropyridine Ca^{2+} channel inhibitors.

increases with frequency of stimulation and membrane depolarization. These workers also found that nitrendipine shows much less use-dependence than verapamil and diltiazem. Nitrendipine blocks the channel not only in the open state but also in its resting state, whereas diltiazem may inhibit Ca^{2+} currents by binding mainly to channels in the inactivated state [26]. The above differences between these agents may be of importance in explaining the basis of tissue selectivity. The greater utility of verapamil over nitrendipine in cardiac arrhythmias is probably due partially to the greater use-dependence of verapamil in some cardiac cells. Verapamil, nitrendipine and diltiazem also prevent outward Cs^+ current through the Ca^{2+} channel [26, 30]; this finding demonstrates that a true channel blockade occurs which is independent of the ion or its direction of flow.

The quaternary ammonium *N*-methyl derivative of gallopamil has been shown to be effective on the inside but not on the outside of the cardiac membrane which indicates that verapamil and related drugs may act from inside the membrane after entering the cell in the uncharged form [31]. The site of action of these drugs appears to be nearer the cytosolic surface [32] than the site at which Cd^{2+} and other inorganic Ca^{2+} channel inhibitors act [33, 34]. Although these drugs do not compete with Ca^{2+} at the cation coordination site where Cd^{2+} binds, they do exhibit "Ca^{2+} antagonism" in the sense that elevated extracellular Ca^{2+} increases the proportion of Ca^{2+} current remaining after drug treatment [26].

Electrophysiologic studies analogous to those of Lee and Tsien, but which measure pure Ca^{2+} current in smooth muscle cells, are required to answer many of the basic questions regarding the selectivity of these drugs for Ca^{2+} channels in certain smooth muscles. This selectivity may be due to (a) the existence of multiple types of Ca^{2+} channels each with different sensitivities to these compounds [35] or (b) the presence of only one type of Ca^{2+} channel [6] whose sensitivity is modified by one or more of the factors [6, 33, 36, 37] known to regulate the state and function of the Ca^{2+} channel. In our view, the simplest hypothesis at this time is that there is one basic type of Ca^{2+} channel whose voltage dependence, sensitivity to Ca^{2+} channel inhibitors, and certain other properties are modified when they become associated with neurotransmitter receptors, or are otherwise regulated by voltage, intracellular Ca^{2+}, protein phosphorylation, or other factors. Thus, it is likely that the sensitivity of different tissues to certain of these drugs will depend on some of the same factors, such as the state of the channel, that determine the varying sensitivity of nerve and cardiac muscle to local anaesthetics [38, 39]. The discrepancies between the potency of these drugs for inhibiting Ca^{2+} current and for binding to isolated membranes may also be explained by modulations in the state of the Ca^{2+} channel that, in turn, alter the affinity of the site that binds Ca^{2+} channel inhibitors.

Specific binding of tritiated Ca^{2+} channel inhibitors

Smooth and cardiac muscle membranes. Several lines of evidence support the view that Ca^{2+} channel inhibitors act at a specific site rather than by non-specific membrane perturbation. In addition to the relative high selectivity of the 1,4-dihydropyridines for Ca^{2+} channels, this evidence includes the stereoselectivity of action and strict structure–activity relationships (for reviews see Refs. 2 and 40). Ligand binding studies have provided further strong support for the view that these agents act at specific high affinity membrane binding sites. For smooth muscles, the evidence suggests that these sites mediate inhibition of contraction. In cardiac muscle, however, further studies are required to establish the relationship between binding and inhibition of contractility.

Binding of [^{3}H]nitrendipine to membranes from both smooth [41–44] and cardiac muscle [45–47] was found to be specific, saturable, rapid, reversible, and stereoselective, and inhibition of radioligand binding by other 1,4-dihydropyridines was found to exhibit a rank order correlation with inhibition of contraction. Binding appears to be to a single set of sites that do not show cooperativity. For guinea pig ileum, nifedipine analogs exhibited an excellent correlation between the potencies for inhibition of K^+-induced contraction and for inhibition of [^{3}H]nitrendipine binding to membranes [42]. The apparent dissociation constants for this series of compounds are the same in membranes from guinea pig ileum [41, 42], bovine aorta [44], and rat [43], dog [45], and rabbit ventricle [46]. Thus, the affinity of the binding site for these drugs is similar in isolated membranes of both smooth and cardiac muscle, whereas the Ca^{2+} channels in intact cardiac cells are much less sensitive to these agents [26, 40]. Binding characteristics for selected membrane preparations are shown in Table 1. For some of these preparations similar data have been obtained using [^{3}H]nimodipine [43, 55–57] or [^{3}H]nifedipine [58]. We have not observed significant differences between these two ligands [43], but others using membranes from guinea pig ventricle have reported that [^{3}H]nitrendipine, in contrast to [^{3}H]nimodipine [56, 57], exhibits a biphasic Scatchard plot.

Most of the data shown in Table 1 were obtained for incubations at low protein concentrations (<100 μg/ml), at 25°, incubated for 60–90 min, at pH 7.0 to 7.4. Similar data have been obtained for membranes from several other smooth muscles [59]. For several preparations the dissociation constant for nitrendipine and nimodipine appears to increase from ≅0.15 nM at 25° to 0.3 to 0.6 nM at 37° [42, 60–62]. Data obtained at very high protein concentrations (>1000 μg/ml) yield K_d values at 25° (≅2 nM) that are 10-fold greater than that observed at lower protein concentrations.

The above discrepancy between binding affinity and potency for negative inotropy suggests that 1,4-dihydropyridine binding sites may be in a different state in intact cardiac cells than in most isolated membrane preparations. Thus, in intact cells, membrane potential, protein phosphorylation or other factors which regulate Ca^{2+} channels [33, 36, 37] may change high affinity binding sites to a lower affinity state. If this occurs, it might also explain some of the cellular selectivity of the Ca^{2+} channel inhibitors. Electrophysiological studies indicate that Ca^{2+} channels in isolated cardiac mem-

Table 1. Characteristics of the binding of [^{3}H]nitrendipine to membranes from various sources

Tissue	K_d (nM)	B_{max} (fmole/mg)	Type of preparation	Ref.
Smooth muscle				
Guinea pig ileum	0.16	1,130	Microsomes	42
Guinea pig bladder	0.15	500	Microsomes	46
Bovine aorta	0.16	80	Sucrose gradient	44
Rat mesenteric artery	0.10	18	Sucrose gradient Purified subfraction	48
Cardiac muscle				
Rat ventricle	0.18	400	Microsomes	43
Dog ventricle sarcolemma	0.14	960	Purified	47
Light sarcoplasmic reticulum		<15	Sucrose gradient Purified subfraction	47
Heavy sarcoplasmic reticulum	0.24	790	Purified subfraction	49
Skeletal muscle				
Sarcolemma	1.8	2,000	Purified	50
Transverse tubules	1.8	50,000	Purified	50
Light sarcoplasmic reticulum	1.5	1,590	Purified subfraction	51
Heavy sarcoplasmic reticulum	1.0	6,720	Purified subfraction	51
Nerve				
Rat cerebral cortex	0.1	102	Total membranes	52
Rat synaptosomes	0.17	114	Synaptosomes	53
Rat pheochromocytoma cell line	1.1	28	Total membranes	54

branes separated for patch clamping are either not functional, or rapidly decay [37]; consequently, binding studies on intact cells are of primary importance. In isolated membranes these channels may be in an inactivated state [63] and this inactivation may convert the binding site from a low to a high affinity conformation. Evidence for both low and high affinity binding sites for [^{3}H]nitrendipine in intact cardiac cells has been reported [64]. The density of the low affinity sites previously reported for isolated cardiac membranes [56] is more than 10-fold greater than the approximately 1–10 sites/μm^2 reported for high affinity binding sites [42, 45, 46] and for the Ca^{2+} channel density in nerve and cardiac cells [36, 37, 65, 66]. There have been no reports of low affinity binding sites in smooth muscle membranes isolated from fresh tissue [48]. Whether or not sites exist that correspond to the lower affinity sites on the putative receptor-operated channel of some smooth muscles can only be determined by future studies.

A large number of drugs that act on neurotransmitter receptors or on other types of channels have been found not to inhibit [^{3}H]nitrendipine binding to smooth [42, 60] and cardiac muscle membranes [46]. However, some interesting exceptions have been found. For example, cyproheptadine, quinacrine, and veratridine all inhibit [^{3}H]nitrendipine binding to guinea pig ileal membranes, whereas chlorpheniramine, pyribenzamine and yohimbine stimulate binding [42]. There is evidence that the effect of some antihistamines on binding of 1,4-dihydropyridines may be related to a direct effect of these compounds on an allosteric binding site common to all non-dihydropyridine Ca^{2+} channel inhibitors [67]. This hypothesis is supported by evidence that verapamil, gallopamil and diltiazem reverse the inhibition by tiapamil or lidoflazine of nitrendipine binding [67]. Calmodulin antagonists inhibit [^{3}H]nitrendipine binding to smooth and cardiac muscle membranes with the same potency as they inhibit calmodulin, suggesting that a similar hydrophobic site may be present in each case [46].

The binding site in smooth and cardiac muscle membranes appears to be a protein whose conformation is dependent on phospholipid. Treatment with heat (60° for 5 min) causes loss of binding, as does protease and phospholipase treatment [42, 46, 57]. Heat sensitivity suggests that the binding is not directly to calmodulin. It is interesting to note that the molecular weight of the multi-subunit binding structure in smooth and cardiac muscle is 275,000 [68], which is similar to that of the Na^+ channel. Furthermore, the Na^+ channel activator, veratridine, is a potent inhibitor of nitrendipine binding, which suggests that the Na^+ and Ca^{2+} channels may contain very similar structures [42]. Support for this idea also comes from the fact that veratridine blocks the Ca^{2+} channel in N1E 115 neuroblastoma cells at the same concentrations that it blocks Na^+ channels [69]. Taken together the above data strongly support the hypothesis that the observed binding to smooth muscle membranes is to the Ca^{2+} channel or to a protein directly associated with the Ca^{2+} channel.

No detailed study has yet been reported on the binding of the 1,4-dihydropyridines to the various pure membrane preparations from smooth muscle, but the available evidence suggests a localization in

sarcolemma [42, 44, 48, 61]. In contrast, contradictory evidence has been obtained for cardiac membranes. Mitochondrial membranes and light sarcoplasmic reticulum [45, 47] were found to be free of high affinity ($K_d = 0.1$ nM) binding sites, and purified sarcolemma from dog [45, 61] and bovine heart [57] was found to be enriched in these binding sites. On the contrary, a detailed subfractionation of canine ventricular membranes indicated that a subfraction of heavy sarcoplasmic reticulum contained a high density of nitrendipine binding sites, and that sarcolemmal markers did not co-purify with these binding sites [49]. Further studies with cardiac membranes may be needed to determine whether this sarcoplasmic reticular fraction is contaminated with transverse tubules.

Skeletal muscle and neuronal membranes: Silent binding sites for nitrendipine? The sensitivity to 1,4-dihydropyridines of Ca^{2+}-dependent contraction of various smooth muscles varies from extremely high (e.g. guinea pig ileum, K^+-induced contraction) to nearly complete insensitivity (norepinephrine-induced contraction of rabbit aorta). A simple explanation for the insensitivity of the latter is that contraction is not acutely dependent on the entry of extracellular Ca^{2+}. The same explanation can be given for the lack of effect of these drugs on twitch contraction of skeletal muscle. Nevertheless, skeletal muscle membranes derived from both transverse tubules [50] and heavy sarcoplasmic reticulum [51] have been reported to contain binding sites for nitrendipine of one-tenth the affinity of those in isolated membranes from other excitable cells. The density of these sites in transverse tubules, as estimated by the number per mg protein, is remarkably high relative to that of cardiac sarcolemma or any smooth muscle membrane preparation studied to date (Table 1). This localization is consistent with the electrophysiological studies indicating that the Ca^{2+} channels in skeletal muscle are concentrated in transverse tubules [70, 71]. As mentioned above for cardiac muscle, it is not known whether the binding sites in the sarcoplasmic reticulum of the skeletal muscle represents contamination with transverse tubules. At high concentrations, nifedipine and gallopamil block Ca^{2+} channels and tonic [72] but not twitch contractions of skeletal muscle [51]. The sensitivity of skeletal muscle to these agents [72], as estimated electrophysiologically, is similar to that of cardiac muscle [26].

There is an interesting similarity between the nitrendipine binding sites in membranes for guinea pig ileal and skeletal muscle membranes in that diltiazem increases the maximal number of binding sites (B_{max}) in both [42, 46, 55]. In membranes from coronary artery [61] and brain [53, 57, 62, 67], diltiazem increases the affinity of the 1,4-dihydropyridine binding sites. A plausible explanation for the increase in B_{max} is that diltiazem converts low affinity binding sites, which are not normally seen in our Scatchard plots, to high affinity binding sites.

As in twitch contraction of skeletal muscle, depolarization-induced influx of $^{45}Ca^{2+}$ in brain synaptosomes is very insensitive to Ca^{2+} channel inhibitors [73, 74], although high affinity binding sites for these drugs are found in brain membranes (Table 1). These binding sites for [^{3}H]nitrendipine are of the same affinity as those of smooth and cardiac muscle, but they show some differences. Although the inhibitions by La^{3+}, Cd^{2+} and other inorganic cations are similar [46, 75, 76], brain membranes lose nitrendipine binding at lower EDTA concentrations than cardiac membranes do [46, 75]. It is interesting to note that Ca^{2+} at millimolar concentrations does not antagonize the binding of nitrendipine but does antagonize channel block by nitrendipine, diltiazem or verapamil [26]. Ca^{2+} in millimolar concentrations has been reported to antagonize [^{3}H]verapamil binding to cardiac membranes [77]. After EDTA treatment, calcium ions at low concentrations [42], as well as many other cations including Mg^{2+}, Ba^{2+}, and Sr^{2+}, tend to restore [^{3}H]nitrendipine binding [75]. The number of [^{3}H]nitrendipine binding sites in the presence of Ba^{2+} has been reported to be much less than in the presence of Ca^{2+}, but the numbers of ion channels estimated electrophysiologically were not different [65, 66]. Therefore, binding to Ca^{2+} channels is not identical to the blocking of Ca^{2+} channels. In other types of neuronal cells (PC12), a correlation between the potency for nitrendipine blockade of $^{45}Ca^{2+}$ influx and its apparent dissociation constant for isolated membranes was found [55]. In contrast to the insensitivity of brain synaptosomes, several neuronal cell lines are highly sensitive to these drugs [54, 78]. It should be noted that a good correlation between the potencies of inhibition of contraction and binding affinity has been seen only for smooth muscle. The binding of [^{3}H]verapamil has also been reported [77] to be of unexpectedly-high affinity ($K_d = 4$ nM) in one report, but of much lower affinity ($K_d = 0.5$ μM) in another study [79]; the lower affinity site may be the α_1-adrenoceptor.

Autoradiographic studies have shown that brain synaptic areas contain high densities of nitrendipine binding sites [80], which is consistent with known electrophysiological evidence for Ca^{2+} channel localization [37]. Studies with isolated membranes also indicate that these binding sites are localized in the synaptosomes [75, 76]. The cerebral cortex, hippocampus, olfactory bulb and striatum were richer in these binding sites than were the midbrain, brainstem or cerebellum. The major conclusions from the above studies on skeletal muscle membranes and synaptosomes are that these membranes contain high affinity [^{3}H]nitrendipine binding sites whose occupation either does not inhibit the Ca^{2+} channels present or has no known functional consequences.

Other sarcolemmal effects

Although inhibition of Ca^{2+} influx appears to be the major mechanism of action of Ca^{2+} channel inhibitors, other sarcolemmal effects have been postulated. For example, nimodipine in low concentrations was found to stimulate the Na^+, K^+-ATPase of certain isolated smooth muscle membranes [81]. Flaim [82] and Hermsmeyer [83] have also proposed that some Ca^{2+} channel inhibitors may stimulate electrogenic pumps. Diltiazem and nifedipine were found to reduce the intracellular Na^+ in blood vessels, which is consistent with the view that these drugs may stimulate the Na^+ pump [82]. However,

reduction of intracellular Na^+ may be caused by the blockade of Na^+ entry through the Ca^{2+} channel. Furthermore, verapamil, diltiazem, and nifedipine in low concentrations produced little or no stimulation of Na^+, K^+-ATPase in isolated membranes from smooth muscle [80]. Although a variety of other mechanisms are also possible, nitrendipine-induced hyperpolarization of vascular smooth muscle may be explained by electrogenic Na^+ or Ca^{2+} pump stimulation [82]. Cinnarizine and flunarizine but not verapamil inhibit Ca^{2+}-ATPase in rat aorta microsomes [84]; it remains to be determined whether these effects are on sarcolemmal or sarcoplasmic reticulum ATPase.

Verapamil and gallopamil, in contrast to the 1,4-dihydropyridines, appear to have many effects on the sarcolemma that are not related to Ca^{2+} channel inhibition. These include inhibition of Na^+ channels [85], certain K^+ channels [28], and several neurotransmitter receptors, including α_1- and α_2-adrenoceptors, and muscarinic and opiate receptors (see Refs. 40 and 86–88). These effects do not show the same stereoselectivity as inhibition of contraction [4, 87, 89].

EVIDENCE FOR INTRACELLULAR SITES OF ACTION

At high concentrations, some Ca^{2+} channel inhibitors stimulate Ca^{2+}-ATPase and Ca^{2+} uptake by isolated skeletal and cardiac muscle sarcoplasmic reticulum [9]. Inhibition of Ca^{2+} release from cytosolic storage sites has been suggested as a possible mechanism of vasodilator action of Ca^{2+} channel inhibitors [7, 88]. Inhibition of Na^+-induced Ca^{2+} release from heart mitochondria was described for relatively low concentrations of diltiazem [90]. Because of the high concentrations of Ca^{2+} channel inhibitors needed to produce some of the above effects, the relevance of these findings to the therapeutic effects of these drugs remains questionable. However, it is known that some of these drugs may reach concentrations in membranes that are ten to twenty times higher than their extracellular levels [86, 91, 92].

It has been proposed that calmodulin represents the site of action of the 1,4-dihydropyridines as well as of other Ca^{2+} channel inhibitors. In the micromolar range, nimodipine and nicardipine were found to competitively inhibit the calmodulin-sensitive and calmodulin-insensitive forms of cyclic AMP phosphodiesterase [93]. The same authors found that verapamil and nimodipine antagonize calmodulin stimulation of phosphodiesterase; the site of verapamil interaction with calmodulin appears to be different than that of trifluoperazine. However, no elevation of either cyclic AMP or cyclic GMP is seen in intact smooth muscle even in the presence of high concentrations of verapamil [94]. Felodipine, another nifedipine derivative, was shown to bind to calmodulin [8] but only at very high concentrations (1 μM). Since the IC_{50} for smooth muscle relaxation by felodipine activity is in the range of 0.1 to 1 nM, it is unlikely that this relaxation is a consequence of calmodulin inhibition. Calmodulin antagonists, e.g. W-7 (*N*-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide), exhibit a much better correlation between calmodulin inhibitory and smooth muscle relaxant activities [95, 96].

Although initial biochemical and physiological studies were consistent with a rather simple relationship between myosin light chain phosphorylation and smooth muscle contraction (for example see Ref. 97), recent studies indicate that more complex mechanisms may operate [98–100]. Maintained contraction of some smooth muscles is not associated with the same increase in Ca^{2+}-dependent myosin light chain phosphorylation as is the initial or phasic concentration [98]. Recent estimates of Ca^{2+} transients using the Ca^{2+} sensitive protein aequorin [101] are also consistent with the view that tension can be maintained with levels of intracellular Ca^{2+} lower than those needed to initiate contraction. Vascular smooth muscle contraction caused by phenylephrine, carbachol and angiotensin results in greater maintained tension for a given amount of light signal than does muscle stimulated by K^+ or histamine [101]. Thus, some stimuli may modify contractile proteins so that they are either more or less sensitive to the increase in intracellular Ca^{2+}. The recorded Ca^{2+} transients as well as the phosphorylation transients for the myosin light chain indicate that a linear relationship between the amount of $^{45}Ca^{2+}$ influx and contraction would be unexpected, particularly for agonist-induced contractions.

The time-courses of myosin light chain phosphorylation observed by Murphy and Gerthoffer [98] suggest that there are at least two separate Ca^{2+}-dependent mechanisms that regulate smooth muscle contraction, one of which may operate mainly during the second phase of contraction. The above results are consistent with previous evidence suggesting that there is not a single common pathway leading to smooth muscle contraction [100, 102, 103]. Although the differential sensitivity of the tonic or maintained phase of contraction is usually thought to be due to differences in the sources of Ca^{2+}, the tonic phase being more dependent on Ca^{2+} influx [1–6], the possibility that such differences are due to separate activation mechanisms at the level of the contractile proteins requires further consideration [103]. Such differences could be a factor in the selective effect of Ca^{2+} channel inhibitors for certain types of smooth muscle stimulated by certain agonists, and for certain phases of contraction.

Additional evidence for intracellular sites of action of diltiazem, verapamil, and nifedipine in cardiac cells comes from measurements of tension and of aequorin light signals. For a given amount of Ca^{2+} signal, the amount of tension was found to be smaller in the presence of these drugs than in their absence [104]. Further studies of this type, particularly for smooth muscle, are needed to examine possible additional effects of Ca^{2+} channel inhibitors on intracellular sites.

PHARMACOLOGICAL BASIS FOR CLINICAL USE OF Ca^{2+} CHANNEL INHIBITORS

The basis for therapeutic use of Ca^{2+} channel inhibitors in the therapy of vascular spasms or hypertension is believed to be the relaxation of smooth muscle caused by reduced Ca^{2+} entry and

consequently decreased free cytosolic Ca^{2+}. The therapeutic success of Ca^{2+} channel inhibitors in the therapy of cardiovascular diseases is based on selectivity of their action for the following.

Ca^{2+} channels

Ca^{2+} channel inhibitors reduce Ca^{2+} entry through Ca^{2+} channels without affecting other mechanisms of sarcolemmal Ca^{2+} transport e.g. Na^{+}–Ca^{2+} exchange or Ca^{2+} leak. Therefore, these drugs do not completely abolish Ca^{2+} entry, so that cellular functions dependent on Ca^{2+} are not inhibited completely. As discussed above, the Ca^{2+} channels of some vascular smooth muscles are more sensitive than those of other smooth muscles.

Organs

Some Ca^{2+} channel inhibitors, e.g. the 1,4-dihydropyridines, reduce Ca^{2+} entry into smooth muscle cells at considerably lower concentrations than into cardiac muscle cells. This selectivity produces vasodilatation without myocardial depression. Most Ca^{2+} channel inhibitors are also relatively selective for smooth muscle as compared to endocrine cells, which also require extracellular Ca^{2+} for their function of hormone release. Although Ca^{2+} channel inhibitors can be shown *in vitro* to reduce the release of many hormones, such effects have not been reported at therapeutic doses *in vivo*.

Vascular beds

Various vascular beds are affected differently by different Ca^{2+} channel inhibitors. This relative selectivity is thought to be based in part on differences in the dependence of a particular vessel on extracellular Ca^{2+} for contraction, and in part to differences in Ca^{2+} channel sensitivity to these drugs. Some vessels that contain larger amounts of Ca^{2+} in the cytosolic storage sites exhibit agonist-induced contraction that is less sensitive to Ca^{2+} channel inhibitors.

Reflex mechanisms

The extent to which an increase in sympathetic nerve activity tends to counteract the vasodilator action of a Ca^{2+} channel inhibitor will differ not only with the degree of drug-induced vasodilatation, but also with the extent of possible direct action of Ca^{2+} channel inhibitors on baroreceptors [105, 106]. The observed decrease in blood flow in the skin of spontaneously hypertensive rats with nitrendipine [107] is likely to be determined by a reflex increase in sympathetic nerve activity. The greater reflex sympathetic activation is assumed to play a significant role in relative ineffectiveness of 1,4-dihydropyridines on cardiac conducting tissue and a similar argument may be made for certain vascular beds [40]. For example, the cutaneous circulation, in contrast to that of the coronary and cerebral circulations, is under major and continuous dominance by the sympathetic nervous system. This sympathetic dominance can be expected to modify the sensitivity of some vascular beds to the Ca^{2+} channel inhibitors.

It was assumed until recently that the basis for selectivity of Ca^{2+} channel inhibitors for certain smooth muscle is the different distribution of Ca^{2+} channels or the existence of different types of channels. The initial ligand binding studies reviewed in this paper have not supported that assumption, although additional studies may still prove this to be true. Thus explanations for the selectivity of the pharmacological action of these drugs may also include: (a) differences in the functional dependence of certain organs or blood vessels on the extracellular calcium, (b) a possibility that modulation of the Ca^{2+} channel may modify specific binding and consequently sensitivity to Ca^{2+} channel inhibitors, (c) the fact that different vascular beds are dominated to a different extent by the sympathetic nervous system, and (d) differences in the relative importance of α_1-, α_2- and γ-adrenoceptors [108] for vasoconstriction of a given vascular smooth muscle cell.

SUMMARY

Ca^{2+} channel inhibitors are viewed as a subgroup of Ca^{2+} antagonists. Most of the currently used Ca^{2+} channel inhibitors are thought to act by reducing Ca^{2+} entry into the cell through Ca^{2+} channels. There is substantial electrophysiological evidence that the major site of action of verapamil, nifedipine and diltiazem in cardiac cells is a sarcolemmal Ca^{2+} channel. Cytosolic sites of action may contribute to their effects but probably only at higher than therapeutic concentrations.

The recent ligand binding studies also tend to support the view that the sarcolemma is the site of action of Ca^{2+} channel inhibitors in smooth muscle. High affinity binding sites for 1,4-dihydropyridines without any established function are found in fast skeletal muscle and some neuronal membranes. The binding of [^{3}H]nitrendipine to membranes from cardiac, skeletal and smooth muscle, and from brain is saturable, reversible and of high affinity; it is sensitive to cations and other drugs that interact with Ca^{2+} channels. Inhibition of [^{3}H]nitrendipine binding and blockade of K^{+} responses in guinea pig ileum by 1,4-dihydropyridines are well correlated, supporting the view that the observed binding is to Ca^{2+} channel. In contrast, blockade of Ca^{2+} channels in cardiac and skeletal muscle and in brain synaptosomes occurs only at higher concentrations than needed to saturate the high affinity binding sites.

The therapeutic success of Ca^{2+} channel inhibitors in the treatment of angina pectoris, hypertension, peripheral vascular diseases, and many other disease entities is based on selective inhibition of Ca^{2+} entry into smooth muscle cells. The specificity of some of these drugs for Ca^{2+} channels in different cell types, organs, or vascular beds is probably determined by receptor modulation and the effect of reflex mechanisms, which in turn determine the indications for their therapeutic use.

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