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COMMENTARY

DIFFERENTIAL SENSITIVITY OF CALCIUM CHANNELS TO DIHYDROPYRIDINES

THE MODULATED RECEPTOR HYPOTHESIS

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Calcium ions are important regulators of a wide range of cellular functions [1]. These include stimulus–response events such as muscle contraction and the release of hormones and neurotransmitters, as well as the regulation of enzyme activities, transport phenomena, and membrane permeabilities. Moreover, calcium has been implicated in the modulation of synaptic efficacy [2] and in the pathophysiology of ischemic cell damage [3]. Many of these actions of calcium are thought to be mediated by the calcium-dependent regulator protein known as calmodulin [4]. Mounting evidence indicates that the role of calcium as an intracellular messenger is as pivotal as that for cyclic AMP [5]. It is clear that further studies of cellular calcium homeostasis and the transduction pathways for the calcium signal are essential for our understanding of cellular physiology.

Calcium concentrations in the cytoplasm are maintained at levels about 10,000-fold less than that of the extracellular fluid. This is accomplished through a number of mechanisms involving both the plasma membrane and internal organelles [6]. Active transport of calcium out of the cell or into intracellular storage sites can occur through ATP-dependent Ca^{2+}-pump activities in the plasma membrane and in internal systems such as the sarcoplasmic reticulum of muscle cells. Plasmalemmal Na^+/Ca^{2+} exchange is an important extrusion mechanism for calcium which relies on the large electrochemical gradient for Na^+ created by the Na^+/K^+-ATPase. Finally, mitochondria are effective calcium buffers when cytoplasmic calcium reaches high levels, accomplishing this buffering ability at the expense of oxidative phosphorylation.

The major route for calcium entry into cells is by diffusion through calcium channels in the plasma membrane [7, 8]. The largest class of calcium channels is the voltage-dependent calcium channels (VDCC) first identified in invertebrate muscle. VDCC have been discovered in almost every excitable membrane studied including invertebrate and vertebrate muscle, axons, nerve cell bodies, nerve terminals, secretory cells, receptor cells, egg cells, epithelial cells, *Paramecium*, and neuroblastoma cells. These calcium currents are blocked by certain polyvalent cations such as Co^{2+}, Ni^{2+}, La^{3+}, and Cd^{2+}, and are able to pass current using Ba^{2+} or Sr^{2+} in place of Ca^{2+}. Many of the VDCC studied, particularly those of vertebrate smooth muscle, are blocked by low concentrations of organic calcium channel inhibitors such as nifedipine, verapamil, and diltiazem [9]. These structurally diverse calcium channel blockers are used clinically for the treatment of a variety of cardiovascular diseases such as angina, hypertension, and certain arrhythmias [10]. Moreover, their potential use in the prophylaxis and treatment of other vascular and nonvascular smooth muscle disorders is under active investigation [11, 12].

It has become apparent from the vast literature that VDCC are more than likely a heterogeneous population of molecular structures with differing properties [7, 8, 13]. This is in contrast to the sodium channel which displays similar properties in a wide range of preparations. Some of the evidence to support the existence of multiple VDCC are: (1) variable potencies of polyvalent cations for blocking calcium currents, (2) different voltage dependencies, ion selectivities, and kinetics of activation and inactivation, and (3) differential sensitivity of VDCC to organic calcium channel blockers. These variations in VDCC properties have been observed between different species and tissues and also within the same preparation. It is the purpose of this review to examine the evidence for the last item of distinction, namely that VDCC differ in their sensitivities to organic calcium channel blockers. In the last several years, a number of laboratories have been actively investigating this issue and have made some very exciting discoveries. These findings, discussed below, suggest that the differences in sensitivity of VDCC to calcium channel blocking drugs may be explained in part by "state-dependent" interaction of these drugs with VDCC. However, the data are far from complete and the controversies still rage.

Biochemical and electrophysiological analysis of voltage-dependent calcium channels. Comparison to receptor binding studies

VDCC have been studied in a variety of preparations including isolated tissue fragments, dissociated cells in culture, cultured cell lines, synaptosomes, and brain slices. VDCC activity in these preparations can be assessed by calcium-dependent physiological responses such as muscle contraction or neurotransmitter release, $^{45}Ca^{2+}$ uptake, or direct electrophysiological analysis using whole cell volt-

age-clamp or patch-clamp techniques. The results of these studies have indicated that VDCC in smooth muscle cells are highly sensitive to low (nanomolar) concentrations of calcium channel blockers, in particular the dihydropyridines (DHP) such as nifedipine, whereas neuronal VDCC are relatively insensitive. On the other hand, cardiac muscle VDCC exhibit an intermediate sensitivity to DHP. Unlike VDCC in brain, certain neuronal cell lines have been found to contain VDCC sensitive to low concentrations of DHP. The recent development of receptor binding assays using tritium-labeled DHP analogs has resulted in the characterization of DHP binding sites in almost every tissue in which VDCC have been identified. The striking similarity in the affinities and pharmacological properties of DHP binding sites in smooth muscle, cardiac muscle, brain, and neuronal cell lines has raised speculation that the differences in DHP sensitivities of VDCC may be more apparent than real. In this section, the properties of VDCC and DHP binding sites are compared.

Smooth muscle. Both vascular and nonvascular smooth muscle are dependent upon a rise in intracellular calcium to initiate muscle contraction. Experimentally, this can be accomplished in two ways: by membrane depolarization using high K^+ solutions or by agonist application (such as noradrenaline). The pathways mediating calcium influx by these two methods have been proposed to involve separate calcium channels, voltage-operated channels (VOC or VDCC) and receptor-operated channels (ROC) [14]. In aorta preparations, high K^+-induced contractions (VDCC) appear to be selectively inhibited by organic calcium channel blockers, although in other vascular and nonvascular smooth muscle this distinction is less prominent [14].

Unlike cardiac muscle (see below), calcium channels in smooth muscle have been studied almost exclusively by the mechanical responses induced by depolarization or agonist stimulation in calcium-containing media. As noted above, these responses are potently inhibited by dihydropyridine analogs with EC_{50} concentrations in the low nanomolar to subnanomolar range [15–17]. Binding studies using [^{3}H]nitrendipine have revealed saturable high-affinity binding sites for DHP in various smooth muscle membranes with K_D values ranging from 0.1 to 4.4 nM and B_{max} values of 18 to 178 fmoles/mg protein [16–20]. In general, the relative and absolute potencies of DHP analogs in displacing [^{3}H]nitrendipine binding have correlated well with their potencies in inhibiting mechanical responses to high K^+ stimulation. For example, Williams and Tremble [18] reported the binding of [^{3}H]nitrendipine to bovine aorta membranes with a K_D of 2.1 nM. In the same preparation, nisoldipine inhibited specific binding with an IC_{50} of 1.5 nM. This is similar to the EC_{50} of 2.4 nM previously reported for nisoldipine inhibition of K^+-induced contractions of rabbit aortic strips [15]. DePover *et al.* [16] described the high-affinity binding of [^{3}H]nitrendipine to pig coronary artery microsomes with a K_D of 1.6 nM. The same investigators found that nitrendipine relaxed pig coronary arteries depolarized by K^+ with an EC_{50} of 1 nM.

The most convincing evidence that DHP binding sites in smooth muscle correspond to VDCC comes from the study by Bolger *et al.* [17] of guinea pig ileal smooth muscle. In this study, the effects of a series of DHP analogs on [^{3}H]nitrendipine binding (K_D = 0.16 nM) to ileal smooth muscle microsomal membranes were compared to their potencies as inhibitors of K^+- or muscarinic receptor-induced contractions. A 1 : 1 correlation was observed for binding activities and inhibition of the tonic contractions induced by high K^+. Regression lines comparing binding activities with inhibition of the phasic component of K^+-induced responses, or with the phasic or tonic components of muscarinic receptor-induced contractions, were shifted from 1 : 1 equivalency such that 10-fold or more higher concentrations were required to block pharmacological responses. Although a number of explanations can be offered to explain these latter observations, the 1 : 1 correlation between [^{3}H]nitrendipine binding and tonic K^+-induced contractions is notable since these contractions are believed to depend almost entirely on the entry of extracellular calcium through VDCC [17].

Cardiac muscle. VDCC in heart muscle have been studied extensively by both direct electrophysiological analysis and by contractile responses of isolated muscle to electrical stimulation. Experiments using these two methods have provided complementary results in that cardiac tissues are about 100-fold less sensitive to the actions of DHP analogs than smooth muscle. Although both absolute and relative potencies vary depending on the region of the heart examined (i.e. atrial vs papillary vs trabecular muscle), EC_{50} values for inhibition of electrically-induced contractions range from 22 to 1000 nM for nisoldipine, nitrendipine, and nifedipine [15, 16, 21]. Similarly, Lee and Tsien [22] found that nitrendipine blocked calcium currents in voltage-clamped single dialyzed ventricular cells with an IC_{50} of 150 nM.

Despite the lower sensitivity of cardiac tissue VDCC to DHP, binding studies have revealed high-affinity DHP binding sites in cardiac membranes with affinities highly comparable to those observed in smooth muscle preparations. K_D values using [^{3}H]nitrendipine have ranged from 0.11 to 6 nM [16, 18, 20, 21, 23, 24], while one study using [^{3}H]nimodipine found a K_D of 1.07 nM [25]. Moreover, as reported for smooth muscle, verapamil partially inhibits DHP binding in an allosteric manner while diltiazem increases receptor binding [20]. These findings indicate that DHP binding sites in cardiac membranes may have no physiological importance or that membrane binding studies do not accurately reflect the *in vivo* properties of cardiac VDCC.

Neuronal tissues. Because of the small size of vertebrate neurons, presynaptic VDCC have been analyzed primarily by neurotransmitter release and $^{45}Ca^{2+}$ uptake into brain slice or synaptosome preparations. On the other hand, direct electrophysiological analysis of postsynaptic VDCC in central and peripheral neurons has been accomplished in a number of laboratories.

Initial studies of K^+-stimulated $^{45}Ca^{2+}$ uptake into rat brain synaptosomes by Nachshen and Blaustein

[26] indicated that neuronal VDCC are relatively insensitive to the actions of organic calcium channel blockers. Thus, verapamil prevented $^{45}Ca^{2+}$ uptake only at high concentrations (100 μM) which affect a number of membrane systems including Na^+ channels [26] and Na^+/Ca^{2+} exchange [27]. In addition, nifedipine at 30 μM had no effect on uptake. Similarly, miniature end-plate potential frequency in depolarized frog neuromuscular junctions was not influenced by 50 μM verapamil [26].

This insensitivity of synaptosomal VDCC to DHP has been reexamined recently by Turner and Goldin [28]. Their studies were based on the premise, first noted by Nachshen and Blaustein [29], that synaptosomal $^{45}Ca^{2+}$ uptake is actually composed of at least two separate components. The first component is a rapid uptake occurring in less than 1 sec which is highly sensitive to block by La^{3+} and is inactivated by predepolarization of synaptosomes. The second slower phase proceeds over a time course of seconds to minutes and is not inactivated by prior depolarization. Turner and Goldin found that the slow phase of uptake could be reduced or eliminated by replacing external Na^+ with choline. Fast phase $^{45}Ca^{2+}$ uptake (1 sec) in Na^+-free medium was inhibited by nitrendipine with a half-maximal effect at 1.7 nM. In Na^+-containing medium, slow phase uptake (10 sec) was inhibited by nitrendipine with a half-maximal effect at 56 nM. It should be noted, however, that maximal inhibition of uptake was less than complete, being 42 and 29% for fast and slow phase uptake respectively.

The reasons for the discrepancies between these findings and the original observations of Nachshen and Blaustein are not readily apparent. First of all, the results of Turner and Goldin suggest that the slow phase $^{45}Ca^{2+}$ uptake into synaptosomes may involve reversal of the Na^+/Ca^+ transporter more than calcium entry through VDCC. If so, the lack of effect of nifedipine on $^{45}Ca^{2+}$ uptake reported by Nachshen and Blaustein might be expected since their measurements were performed after 2 min of depolarization in high K^+ solution. This would also explain the small percent inhibition of slow phase uptake (measured in Na^+-containing medium at 10 sec) by nitrendipine reported by Turner and Goldin. Daniell *et al.* [30] found a similar insensitivity of slow phase uptake to DHP measured in Na^+-containing medium at 3 sec of depolarization.

Confirmation of synaptosome data would be aided by direct electrophysiological analysis of VDCC in vertebrate neurons. Although postsynaptic VDCC have been characterized in several central and peripheral neuron systems, their sensitivities to DHP have not been well characterized. Nevertheless, receptor binding studies in brain synaptosome and membrane preparations have revealed high-affinity DHP binding sites almost identical in properties to those observed in smooth and cardiac muscle. K_D values for these brain binding sites have ranged from 0.086 to 1.0 nM for [^{3}H]nitrendipine [20, 24, 28, 31, 32] and 0.62 nM for one study employing [^{3}H]nimodipine [33]. The high degree of similarity between DHP binding sites in brain and other tissues suggests that VDCC in brain are indeed modulated by low concentrations of DHP under appropriate conditions, as described by Turner and Goldin. However, in analogy to cardiac tissue, there is still a 5-fold difference in the ability of nitrendipine to block fast phase calcium uptake (EC_{50} = 1.7 nM) and the K_D for [^{3}H]nitrendipine binding sites in the same preparation (K_D = 0.36 nM) [28].

Clonal cell lines. Neuronal cell lines have provided a convenient and homogeneous system in which to study membrane channels. A number of clonal cell lines have been found to contain VDCC as evidenced from electrophysiological experiments and studies of depolarization-induced $^{45}Ca^{2+}$ uptake. In general, VDCC in these cells are uniformly sensitive to blockade by nanomolar concentrations of DHP. For example, Freedman *et al.* [34] found that high K^+-induced $^{45}Ca^{2+}$ uptake into the neuroblastoma × Chinese hamster brain hybrid (NCB-20) could be inhibited by nisoldipine, nimodipine, nitrendipine, and nifedipine with IC_{50} values of 0.58, 5.6, 6.4, and 9.2 nM respectively.

The clonal cell line PC12, derived from a rat pheochromocytoma, has been employed extensively as a model system for studying excitation–secretion coupling since these cells exhibit calcium-dependent, depolarization-induced secretion of catecholamines, ATP, and acetycholine. High K^+-induced $^{45}Ca^{2+}$ uptake into these cells is inhibited by nicardipine with an IC_{50} of 20 nM [35]. A very similar IC_{50} of 15 nM was found for nicardipine inhibition of high K^+-stimulated ATP release in these cells [36]. BayK 8644 is a novel dihydropyridine analog that, in direct contrast to other DHP, exhibits vasoconstrictive and positive inotropic actions [37]. Thus, this new compound has been termed a "calcium channel agonist". In PC12 cells, Bay K 8644 enhanced the high K^+-stimulated release of [^{3}H]noradrenaline in nanomolar concentrations, an effect blocked by nitrendipine [38].

Several laboratories have simultaneously studied the effects of DHP on VDCC and the properties of DHP binding sites in PC12 cells. Toll [39] found that the affinities of DHP and other calcium channel blockers for [^{3}H]nitrendipine binding sites correlated very well with their potencies in inhibiting depolarization-induced $^{45}Ca^{2+}$ uptake. [^{3}H]Nitrendipine binding to PC12 membranes exhibited a K_D of 1.1 nM and a B_{max} of 27.5 fmoles/mg of protein. Albus *et al.* [38] found similar kinetic constants for [^{3}H]nitrendipine binding to PC12 membranes and reported that BayK 8644 inhibited binding with a K_i of 16 nM. This was similar to the EC_{50} of 10 nM for stimulation of [^{3}H]noradrenaline release by BayK 8644 in the presence of 25 mM K^+. These results are consistent with the hypothesis, first supported from smooth muscle studies, that DHP binding sites represent functional receptors through which DHP modulate calcium channel activity.

Intuitively, it would appear that high-K^+-stimulated $^{45}Ca^{2+}$ uptake into neuronal cell lines represents a process identical to that in rat brain synaptosomes. However, several findings indicate that these two processes differ. First of all, $^{45}Ca^{2+}$ uptake into cell lines is potently and completely inhibited by DHP analogs, when measured at 2–10 min following depolarization in high K^+ medium. Uptake into synaptosomes measured at similar times is insensitive

to DHP. On the other hand, fast phase (1 sec) $^{45}Ca^{2+}$ uptake into synaptosomes is effectively blocked by DHP. Whether neuronal cell lines possess fast and slow phases of depolarization-induced $^{45}Ca^{2+}$ uptake has not been addressed. La^{3+} is a potent blocker of $^{45}Ca^{2+}$ uptake in synaptosome preparations [29] but was found to increase $^{45}Ca^{2+}$ uptake in the NCB-20 cell line [34].

The exact reasons for these discrepancies are not clear. However, differences in kinetics of inactivation of VDCC, contribution of Na^+/Ca^{2+} exchange processes, and the presence of multiple calcium channel subtypes may underlie the experimental observations. Moreover, VDCC in these clonal cell lines may be influenced by their state of differentiation in culture [34]. Interestingly, Ogura and Takahashi [36] reported that nicardipine inhibits high-K^+-stimulated $^{45}Ca^{2+}$ uptake into PC12 cells but has no influence on the Ca^{2+}-dependent action potential elicited in these cells. The authors suggest that PC12 cells contain two types of VDCC: a DHP-insensitive spike-generating channel and a slowly-inactivating DHP-sensitive channel. Evidence for two types of VDCC differing in their inactivation kinetics has been provided from voltage-clamp studies of NG108-15 cells [40].

The modulated receptor hypothesis

A fundamental question raised by the studies described above is whether the differential sensitivity of VDCC to organic calcium channel blockers is real or can be accounted for by experimental differences. The results of DHP binding studies suggest that the latter is the case, since DHP binding sites display a high degree of homogeneity between preparations. Moreover, it is clear that VDCC, like other voltage-dependent membrane channels, are dynamic molecular entities that are in constant transition between various states (resting, open, inactivated), depending on the membrane potential. Receptor binding studies in membranes, however, identify channels that presumably are in a static inactivated state since the potential difference across the membranes is lost.

Recently, Sanguinetti and Kass [41] and Bean [42] have obtained experimental evidence to support the hypothesis that organic calcium channel blockers exert their effects on VDCC in a state-dependent manner. Their findings are best explained using the Modulated Receptor Hypothesis (MRH) first developed by Hille [43] and Hondeghem and Katzung [44] to account for the actions of local anesthetic drugs on voltage-dependent Na^+ channels. According to the hypothesis (see Fig. 1), charged drug forms can only reach their channel binding site via a hydrophilic pathway, available only when the channels are open. Neutral, lipid-soluble drug forms can reach the binding site via a hydrophobic pathway which is available at all times. Thus, ionized drugs are obliged to bind and unbind to open channels (O-O*), while neutral forms can also interact with rested (R-R*) and inactivated channel configurations (I-I*).

The study by Sanguinetti and Kass [41] examined the voltage- and use-dependency of VDCC block by organic calcium channel blockers in calf Purkinje fibers. The authors found that block by verapamil developed only after repetitive pulsing to positive

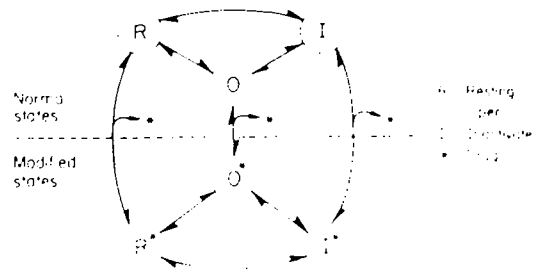

Fig. 1. Schematic representation of the Modulated Receptor Hypothesis. Reproduced from Hille [43], *The Journal of General Physiology*, 1977, Vol. 69, p. 508, by copyright permission of the Rockefeller University Press.

potentials (to maximize exposure of open channels to the drug), whether the membrane holding potential was −70 or −45 mV. This is consistent with previous studies and with the MRH since verapamil ($pK_a = 8.7$) is approximately 95% charged at physiological pH. On the other hand, nisoldipine and nitrendipine, neutral drugs at physiological pH, exhibited considerably less frequency-dependent inhibition of calcium currents. Block by these compounds (using low frequency pulses) was enhanced markedly by a change in holding potential from −70 to −45 mV. In addition, these drugs caused a hyperpolarizing shift in the steady-state inactivation curve measured with long 30-sec prepulses. These findings indicate that nisoldipine and nitrendipine have a strong affinity for I channel states over R states. Moreover, evidence for O state block was obtained from the observed increase in apparent inactivation of calcium currents and the small but significant increase in channel block at high frequency stimulation. Finally, additional evidence to support the MRH was provided by experiments using nicardipine, where the use-dependency of channel block was found to be intermediate between verapamil and nisoldipine, consistent with the intermediate pK_a of nicardipine.

Bean [42] obtained qualitatively similar results in his studies of single canine ventricular cells using the whole cell patch clamp technique. In these experiments, nitrendipine block of VDCC was found to depend strongly on the holding potential. Concentration–response curves provided K_D values for nitrendipine block of 0.36 and 760 nM for holding potentials of −10 and −80 mV respectively. Since VDCC are >2/3 inactivated at −10 mV compared to −80 mV in this preparation, Bean interpreted these findings as demonstrating a high-affinity of nitrendipine for binding to I channel states. The same conclusion was reached by Sanguinetti and Kass.

The data above provide a simple and convincing explanation for the discrepancies between DHP binding studies and the potencies of DHP for blocking VDCC in cardiac preparations. Thus, the K_D of 0.36 nM from nitrendipine block of VDCC in depolarized cells closely approximates the K_D for [^{3}H]nitrendipine binding (0.11 to 6.0 nM) observed in cardiac membrane preparations. These membrane fractions, prepared after cellular disruption, sense a

depolarized membrane potential (zero) and VDCC can be expected to be completely in the I configuration, the high-affinity state for nitrendipine.

The MRH may also explain the close correlation observed in smooth muscle between DHP binding and DHP block of mechanical responses to stimulation. First of all, the resting potential of smooth muscle preparations may approach values more positive than −60 mV [45]. Second, high K^+-stimulation of smooth muscle contraction is usually measured over a period of minutes during which DHP binding to inactivated VDCC is optimized. Thus, smooth muscle represents a system in which high-affinity binding of DHP to I states of VDCC is facilitated. In contrast, cardiac tissues generally maintain a more negative resting potential, and stimulation paradigms for measuring contractile responses employ low-frequency, short-duration electrical pulses that do not promote I channel transitions [15]. Consequently, pharmacological responses are some 100-fold less sensitive to DHP than are DHP binding sites in cardiac membranes.

It remains to be determined whether the MRH can account for the effects of DHP on VDCC in brain synaptosomes and in neuronal cell lines. In synaptosomes, Turner and Goldin [28] found high-potency block of fast phase $^{45}Ca^{2+}$ uptake by nitrendipine with an EC_{50} of 1.7 nM. This is several-fold higher than the reported K_D values for [^{3}H]nitrendipine binding in brain membrane and synaptosome preparations. However, the true potency of this drug may be obscured by the fact that less than half of the $^{45}Ca^{2+}$ uptake could be blocked. Since high-affinity channel block by DHP was found to exhibit slow kinetics ($t_{1/2}$ of several minutes) in depolarized cardiac cells [42], the potency of DHP in synaptosomes may be underestimated since Ca^{2+} uptake was measured at 1 sec of depolarization. Conversely, the fact that some VDCC block by DHP is demonstrable in synaptosomes (which maintain a fairly negative resting potential) may be attributed to the use of 1-sec depolarizations, during which time a significant portion of channels may reach the I state necessary for high-affinity DHP binding. The close correlation between DHP binding and inhibition of $^{45}Ca^{2+}$ uptake by DHP in PC12 cells may be analogous to the case of smooth muscle. Thus, high-K^+ depolarizations over a period of minutes was used to assess the effects of DHP on VDCC in these cells [39].

Dihydropyridine-resistant calcium channels

Recent work from a number of laboratories has identified a new class of VDCC with properties distinct from the more common VDCC with which they coexist [46–53]. These novel VDCC are activated in a relatively negative voltage range, inactivate within tens of milliseconds, and conduct barium and calcium equally well. Their properties are similar in a number of preparations including chick [49] and rat [47] sensory neurons, GH3 cells [46], canine atrial cells [51], guinea pig ventricular cells [48], and neuroblastoma cells [52, 53].

The most intriguing feature of the novel VDCC is their insensitivity to pharmacological modulation. Unlike the more well-studied VDCC, these fast-inactivating VDCC are resistant to block by nimodipine and nitrendipine and are unaffected by the "calcium agonist" Bay K 8644 [48, 49, 51]. Similarly, these currents are less sensitive to the effects of isoproterenol [51] and verapamil [47, 52]. The resistance of fast-inactivating VDCC in heart cells to nitrendipine block was uninfluenced by changes in holding potential [51], indicating the lack of high-affinity binding to these channels whether in the resting or inactivated state. It will be interesting to see if fast VDCC can be modulated by other pharmacological agents or neurotransmitter substances.

Conclusions

In summary, the Modulated Receptor Hypothesis has gained experimental support to explain the differential sensitivity of VDCC to organic calcium channel blockers. However, additional studies will be necessary to substantiate its applicability. Most importantly, the MRH needs to be tested in voltage-clamp experiments of smooth muscle, neurons, and clonal cell lines. In addition, a wider range of calcium channel blocking compounds needs to be examined to determine the influence of pK_a, lipid solubility, and molecular size on the blocking characteristics of these drugs. Notably, VDCC with properties distinct from the conventional VDCC have been found in a variety of tissues [46–53] and appear to be insensitive to organic calcium channel blockers. Nevertheless, the initial findings of Sanguinetti and Kass [41] and Bean [42] have helped to clarify many of the controversies surrounding DHP binding sites. If these sites do represent functional calcium channels, then the isolation and reconstitution of VDCC may be accomplished in the imminent future.

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