Block of L-Type Calcium Channels by Charged Dihydropyridines

Sensitivity to Side of Application and Calcium

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ABSTRACT We have studied block of L-type calcium channels by intracellular and extracellular application of the ionized dihydropyridine derivatives amlodipine and SDZ 207-180. We find that extracellular application of either drug causes voltage-dependent block of calcium channels. However, neither drug is effective when applied intracellularly. The insensitivity of calcium channels to intracellular drug is not due to the low concentrations of cytosolic calcium, because voltage-dependent block by ionized amlodipine, SDZ 207-180, and the neutral drug nisoldipine persists under conditions in which Ca0 is buffered by EGTA. In fact, the time course of the development of block by the ionized but not neutral drug molecules studied, is slower in the presence than in the absence of calcium. Our results indicate that the DHP binding site of the L-type calcium channel is close to the extracellular surface of the cell membrane and that ionized DHP molecules may interact with the receptor in a manner that is uniquely affected by calcium.

INTRODUCTION
Specific 1,4-dihydropyridine (DHP) ligands have been used as key molecular probes of L-type calcium channels and as powerful therapeutic tools to treat a wide range of cardiovascular disorders (reviewed by Janis and Triggle, 1990). These compounds have been used to isolate and purify the channel protein (Flockerzi et al., 1986; reviewed by Catterall, 1988; Høsey and Lazdunski, 1988). DHP's bind with high affinity to the α1-subunit of the DHP receptor protein (Galizia et al., 1986; Sharp et al., 1987; Sieber et al., 1987). However, although the primary structures of rabbit skeletal and heart muscle DHP receptors have been cloned (Tanabe et al., 1987; Mikami et al., 1989) and functional activity of the L-type calcium channel has been expressed (Tanabe et al., 1988; Mikami et al., 1989; Perez-Reyes et al., 1989) from the cloned cDNAs, the molecular identity and location of the DHP binding site have not yet been determined.

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We have shown previously that the charged form of amlodipine, a tertiary DHP molecule, is useful as a probe for the location of the DHP binding site in the cell membrane (Kass and Arena, 1989). In the present investigation we have used a combination of charged and neutral DHP compounds to test whether the DHP binding site, like that for phenylalkamines, is accessible from the inner surface of the cell membrane. We have determined whether block of monovalent ions, like divalent ions, is voltage dependent; and, additionally, have investigated the dependence of channel block on the absence or presence of calcium.

METHODS

Single ventricular myocytes were isolated from either ventricle of adult guinea pigs using a method similar to that of Mitra and Morad (1985) which has been previously described (Arena and Kass, 1988).

Recording methods were as described by Hamill et al. (1981) for the whole cell configuration. Patch pipettes were made from Gold Seal Accu-fill 90 Micropets (Clay Adams, Inc., Parsippany, NJ). The resistance of the pipettes was typically 1–3 MΩ when filled with 140 mM CsCl. Series resistance compensation was used in all experiments and was adjusted to give the fastest possible capacity transients without producing ringing. Membrane currents were measured with and voltage was controlled by either a Yale Mark IV (fabricated in this laboratory from parts available at New Haven, CT) or an Axopatch 1C (Axon Instruments, Inc., Foster City, CA) voltage clamp. Cell capacitance was determined directly by capacity compensation of these circuits. Data were sampled once every 0.3 ms and filtered at 1–2 kHz with an eight pole Bessel filter (Frequency Devices, Inc., Haverhill, MA). In experiments designed to dialyze cells with drug-containing solutions, pipette resistances were chosen to be ≤2 MΩ and drugs were added to the intracellular solutions at the concentrations indicated for each experiment.

Solutions and Drugs

Solutions were chosen to eliminate K channel currents. Thus, the standard pipette solution contained in millimolar: 60 CsCl, 50 aspartic acid, 68 CsOH, 1 MgCl₂, 1 CaCl₂, 11 ethylene-glycol-bis-N,N',N'',N'''-tetraacetic acid (EGTA), 5 K₆ATP, 10 HEPES (pH 7.4). The standard bath solution contained: 130 mM NaCl, 4.8 CsCl, 5 glucose, 5 HEPES (pH 7.4). In experiments designed to measure divalent ion currents, 2 mM MgCl₂ and divalent charge carriers at indicated concentrations were added to the basic solution. In experiments designed to measure monovalent currents the following were added to the basic extracellular solution: 2 EGTA plus 50 μM MgCl₂ (for the measurement of Na currents) or 2 ethylenediaminetetraacetic acid (EDTA) and 500 μM MgCl₂ (for the measurement of outward currents). Sodium channels were eliminated by 10–50 μM tetrodotoxin (TTX) (Behring Diagnostics, LaJolla, CA) and by replacement of Na by N-methyl-d-glucamine in some experiments.

The structures of the three DHP compounds used in this study are shown in Fig. 1. Nisoldipine and SDZ-207-180 were dissolved in polyethylene glycol 400 (PEG) to make a concentrated (1 mM) stock and diluted in the bath to the final concentration. PEG at the concentration used (<0.01%) has been shown to have no effects of its own on Ca channel currents (Kass, 1982). Amlodipine was dissolved in water as a concentrated (1 mM) stock solution. Amlodipine concentrations were chosen as previously described (Burges et al., 1985, 1987; Kass and Arena, 1989). SDZ-207-180 was a gift of Sandoz, Ltd., Basle, Switzerland; nisoldipine was a gift from Miles Laboratories, New Haven, CT; and amlodipine was a gift from Pfizer Central Research, Sandwich, UK.
Voltage Protocols

Membrane potential regulates DHP modulation of \( I_{\text{Ca}} \); block is promoted at positive voltages and relieved at negative voltages (for example, see Sanguinetti and Kass, 1984). The time course of the development or removal of block can be determined by applying a series of test voltages from different holding potentials. In the present study voltage protocols described in detail and illustrated in Kass and Arena (1989) were used to determine onset and recovery of block. Drug onset was measured by applying a depolarizing "train" protocol in which the holding potential was changed from \(-80\) to \(-40\) or \(-50\) mV and test voltage pulses were applied once every 5 s. Test voltages were either 0 mV (Na\(^+\) currents), +10 mV (Ca\(^{2+}\) and Ba\(^{2+}\) currents), or +40 to +60 mV (Cs\(^+\) and K\(^+\) currents) and test pulse duration was 200 ms. This protocol was previously shown to maximize the onset of voltage-dependent block by ionized DHP compounds (Kass and Arena, 1989). After development of block recovery was measured using train protocols in which the holding potential was changed from \(-40\) to \(-80\) mV and pulses were applied to the same test potentials, but test pulse duration was either 20 or 40 ms to minimize pulse-induced inactivation.

In experiments that required recording currents from potentials negative to \(-60\) mV, 50–100-ms prepulses were applied to \(-40\) mV to inactivate sodium channel and T-type calcium channel currents (Bean, 1985; Marchetti and Brown, 1988). Thus, in this paper current referred to as Ca channel current \( (I_{\text{Ca}}) \) corresponds to L-type Ca channel current according to the terminology suggested by Nilius et al. (1985).

DHP Receptor: Agonism and Antagonism

Most DHP compounds are capable of causing enhancement or block of \( I_{\text{Ca}} \) depending on cell membrane potential (Hess et al., 1984; Sanguinetti et al., 1986; Kass, 1987). Binding and
electrical data have provided evidence that these effects are due to interactions with more than one binding site (Brown et al., 1986; Kokubun et al., 1986; Wei et al., 1986; Williams et al., 1985; Hamilton et al., 1987). The binding site probed in this study is associated with inhibition of channel activity. We rarely measured agonistic responses to SDZ 207-180 applied under conditions of negative (−80 to −100 mV) holding potentials, but under similar conditions observed enhancement of currents by amlodipine at pH 7.4 in ~30% of our experiments. Further investigations will be needed to test whether electrical evidence with charged DHP derivatives can support the view that distinct binding sites exist for agonism and antagonism of L-channels.

Curve Fitting and Statistical Procedures

Where appropriate, experimental data were fitted with functions of one or two exponentials plus an arbitrary baseline using procedures previously described (see Sanguinetti and Kass, 1984). All data in the text and figures are means ± SEM. T-tests were used to determine statistical significance between groups (Rosner, 1986). Multiple comparisons were made using the pooled estimate of variance from a one-way ANOVA.

RESULTS

The DHP Binding Site Is Accessed from the Outside but Not the Inside of the Cell

To test for an intracellular location of the DHP binding site, we measured the sensitivity of calcium channel current to internal and external application of the permanently charged drug SDZ-207-180. The charge on this compound will restrict it from diffusing out of the cell across the lipid membrane (Hille, 1977). As a control for internal dialysis, we first measured the effects of internally applied D890, a permanently charged phenylalkamine derivative that has been shown to block I_{c,a} from the inside of cardiac cells (Heschler et al., 1982; Lee and Tsien, 1984). We used pulse-dependent block during train protocols to assay drug activity and, as shown in Fig. 2 A, there is a progressive increase in block as this compound diffuses into the cell. In contrast, we found that SDZ 207-180 had little effect when applied internally. This can be seen in Fig. 2 B, which compares pulse-dependent block after 20 min of dialysis with 10 μM intracellular SDZ 207-180 to a 3-min exposure to 200 nM SDZ 207-180 applied externally to the same cell. We found a similar pattern when we compared intracellular with extracellular application of ionized amlodipine (data not illustrated): currents were only blocked by extracellularly applied drug.

Because of the large size of isolated heart cells, we were concerned that diffusional exchange between the cell interior and patch pipette might be limited within the time frame of our experiments. Rates of diffusional exchange can be estimated for heart cells (C_e ~100 pF) by the methods of Pusch and Neher (1988). For pipette resistances on the order of 3 MΩ (typical for these experiments), and for a molecular mass of 350 D, the diffusion time constant is 15 min. To confirm the insensitivity of I_{c,a} to internal application of SDZ 207-180, we compared our results obtained in heart cells with experiments carried out in GH_{4}C_{1} pituitary tumor cells. These cells are small (mean C_e = 12.65 ± 1.2 pF, N = 12) and the predicted diffusional time constant is 40 s (Pusch and Neher, 1988). For these cells, we found that internally applied D890 (100 μM) completely blocked I_{c,a}, consistent with the predictions of Pusch and Neher. However, as was the case for heart cells, I_{c,a} was not blocked by
FIGURE 2. Effects of internal and external application of quaternary Ca channel blockers in heart cells. Currents were measured during the application of onset train protocols with permanently charged drugs added to the whole-cell patch pipettes. (A) D890 (100 μM) was included in the pipette. Currents were measured during onset trains applied 5, 10, and 15 min after establishing whole-cell recording conditions. Currents at start of each train were 954, 871, and 754 pA, respectively. Cell 26081. Charge carrier: 2 mM Ca. (B) SDZ 207-180 was (1 μM) added to pipette and no pulse-dependent block developed. The solid circles are currents measured during an onset train applied 23 min after establishing whole-cell conditions. The cell was then exposed to an external solution containing 200 nM SDZ 207-180. The solid triangles are currents recorded in response to the same onset train protocol 3 min after changing to this external solution. Cell 11081.
internal SDZ 207-180 in GH4C1 cells, but was blocked by external drug application (Fig. 3). Fig. 4 summarizes the results for internal vs. external application of the quaternary DHP for both types of cells.

These experiments provide evidence that ionized drug access of the DHP receptor is not via the inner surface of the cell membrane. However, because intracellular calcium is buffered to submicromolar levels in our experiments (Methods), it is possible that the charged DHP molecule is not an effective blocker under these conditions.

% BLOCK

0 20 40 60 80 100

INTERNAL EXTERNAL

Figure 4. Block of calcium channel currents by external, but not internal, application of SDZ 207-180 in heart and GH4C1 cells. The bars summarize the mean current blocked by internal and external application of SDZ 207-180 in heart (open bars) and GH4C1 cells (shaded bars). Internal concentrations were 1–10 μM in heart (n = 6) and 500 nM in GH4C1 cells (n = 9). External concentrations were 200 nM in heart (n = 7) and 500 nM for GH4C1 cells (n = 8). (*) Significantly greater than internal application P ≤ 0.005.
conditions. We tested for this possibility by measuring the influence of calcium on the voltage dependence of SDZ 207-180 block.

Influence of Calcium on DHP Block and Recovery: Contrast between Neutral and Charged Drugs

Fig. 5 shows examples of experiments in which SDZ 207-180 block was studied under conditions in which calcium carried the charge and in which \( \text{Ca}_0 \) was buffered and Na or Cs were the charge carriers. Records are shown in the absence of drug, and during the onset of SDZ 207-180 block of currents. This compound blocks in a voltage-dependent manner when calcium is the charge carrier, and when \( \text{Ca}_0 \) is buffered and

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**Figure 5.** Influence of calcium on voltage-dependent block by SDZ 207-180. Current traces illustrate Ca, Na, and Cs ions carrying the charge during onset train protocols in the presence of 200 nM SDZ 207-180. Traces are shown at the start of the train protocol, 50 s, and 100 s after applying pulses (Na, Cs), and 100 and 200 s after starting the onset train (Ca). Monovalent currents were measured in the presence of EGTA and EDTA (Methods). The plot shows normalized currents measured in the absence (open symbols) and presence (solid symbols) of 200 nM SDZ 207-180 as a function of time after start of the train protocol. The permeant ions were Ca (squares), Cs (circles), and Na (triangles). The tonic block at start of train protocols for each charge carrier was 13% (Ca), 0% (Na), and 14% (Cs). Calibration bars: 18 ms; 400 pA (Ca), 1.3 nA (Na), and 3.14 nA (Cs). Cells: 11391 (Ca); 111491 (Na); 22AG94 (Cs).
Cs\(^+\) or Na\(^+\) carry the charge. Interestingly, the development of block is faster when Ca\(_0\) is chelated by EGTA. Similar results were obtained with ionized amlodipine. Thus, the ineffectiveness of internally applied SDZ 207-180 and amlodipine is not due to the low concentration of calcium in our intracellular solutions.

Neutral Drugs: Nisoldipine

Do neutral and charged DHP compounds share a common sensitivity to conditions in which Ca\(_0\) is chelated? We investigated the influence of Ca\(_0\) on the voltage-dependence of block by the neutral DHP nisoldipine to test this possibility and the
results are presented in Fig. 6. Under conditions in which Ca\textsubscript{0} is chelated, block of monovalent currents by nisoldipine is voltage-dependent, and the time course of block onset is affected neither by the marked difference in test pulse voltage nor in the ionic species carrying the charge. However, in contrast to the actions of the ionized molecules, the onset of nisoldipine block is unchanged when Ca\textsubscript{0} is not buffered and calcium is the charge carrier.

**DISCUSSION**

The major new result presented in this paper is that the permanently charged compound SDZ 207-180 and ionized amlodipine are much more potent when applied extracellularly compared with intracellular application. In addition, we find that the development of voltage-dependent block by ionized and neutral DHP molecules persists under conditions when calcium is buffered to micromolar concentrations, but that the onset of block by the two ionized drugs is slower in the presence than in the absence of calcium.

*Extracellular Access to the DHP Binding Site*

Our experiments in which we compared intracellular and extracellular application of ionized amlodipine and SDZ 207-180 in heart and GH\textsubscript{4}C\textsubscript{1} cells provide evidence that these two charged DHP compounds cannot reach the DHP receptor via an intracellular pathway. This finding contrasts with the accessibility of the permanently charged drug D890 to the phenylalkamine receptor which occurs via an intracellular pathway in guinea pig ventricular cells (Heschler et al., 1982), but not in vascular smooth muscle cells of the rabbit (Leblanc and Hume, 1989). Our result is, however, consistent with data reported by Iijima et al. (1984) for intracellular application of the DHP nicardipine. The difference in intracellular accessibility of the two permanently charged drug types supports the view that the binding sites for DHPs and phenylalkamines on the \( \alpha_\text{i} \)-subunit of the DHP receptor protein are physically separate entities (Glossmann et al., 1984).

*Location of the DHP Binding Site*

Valdivia and Coronado (1990) have reported that SDZ 207-180 can interact with a high affinity DHP binding site when applied to either side of a planar lipid bilayer into which skeletal muscle transverse tubule Ca channels had been incorporated. They suggested that the 10 methylene groups that separate the quaternary ammonium from the DHP ring in this molecule (Fig. 1) are sufficiently long to allow the DHP moiety to be uncharged and reach the DHP binding site through the lipid bilayer regardless of the side of application. If this view is correct, then our results, which show an asymmetrical response in both intact heart and GH\textsubscript{4}C\textsubscript{1} cells, suggest that the high affinity DHP binding site is closer to the extracellular membrane face than the intracellular face and is just beyond the reach of the methylene spacer chain when applied from the intracellular side of the membrane. These results are consistent with conclusions reached from investigations of the sensitivity amlodipine to changes in external hydrogen ion concentrations (Kass and Arena, 1989).
Calcium Ions Modify Block by Ionized DHP Derivatives

Our results cannot be explained by a change in blocking activity of SDZ 207-180 due to the low levels of intracellular calcium because we find that voltage dependence of block by neutral and ionized DHP molecules is preserved under conditions in which calcium is buffered to micromolar concentrations by either EGTA or EDTA (see also Hess et al., 1986; Hadley and Hume, 1987). However, under conditions in which calcium is the permeant ion, block by ionized amlodipine and SDZ 207-180 develops at a slower rate than under conditions when Ca\textsubscript{0} is buffered and monovalent ions carry the charge. Block by the neutral molecule nisoldipine is not affected by chelating Ca\textsubscript{0}. Recovery from block by these two ionized DHPs, but not nisoldipine, is also slower when calcium is the charge carrier (data not shown).

These results are similar to those of Carbone and Lux (1988a, b, 1989) who reported that \(\omega\)-Conotoxin (\(\omega\)-CTX) blockade of high threshold (L-type) calcium channels differed markedly depending on the type of ion carrying charge through the open channel pore. Those authors suggested that \(\omega\)-CTX binding was influenced by charge carrier–induced channel conformational changes. Permeating charge species–induced differences in channel conformation are expected since channel permeation is determined by two intrapore binding sites with varying affinity for monovalent and divalent ions (Almers and McCleskey, 1984; Hess et al., 1986). In fact, Prod'hom et al. (1989) have reported that charge carrier–induced conformational changes underlie the modulation of proton-induced fluctuations in L-channel conductance levels, and suggested that Ca forms a stabilizing complex with the calcium channel protein and limits the flexibility of the channel protein that exists when other ion species permeate the channel. Our results are consistent with this view of the channel, but cannot rule out the possibility that it is an interaction between the charged head groups of amlodipine or SDZ 207-180 with extracellular calcium that interacts with the drug-bound state of the channel. Future experiments with other ionized drug molecules will be useful in distinguishing these possibilities.

SUMMARY

In summary, our results have shown that charged DHP derivatives are very useful as probes of the L-type channel in intact cell membranes. We have found that ionized amlodipine and the permanently charged derivative SDZ 207-180 are useful in determining the location of the DHP binding site that causes inhibition of channel activity. Studies of other DHP derivatives with different charge locations will further define the location of this site and provide insight into possible conformational changes L-channels undergo for varied permeant ions.

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REFERENCES

Almers, W., and E. W. McClesky. 1984. Non-selective conductance in calcium channels of frog muscle: calcium selectivity in a single-file pore. Journal of Physiology. 353:585–608.

Arena, J. P. and R. S. Kass. 1988. Block of heart potassium channels by clofilium and its tertiary analogs: relationship between drug structure and type of channel blocked. Molecular Pharmacology. 34:60–66.
Bean, B. P. 1985. Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity, and pharmacology. Journal of General Physiology. 86:1–30.

Brown, A. M., D. L. Kunze, and A. Yatani. 1986. Dual effects of dihydropyridines on whole cell and unitary calcium currents in single ventricular cells of guinea-pig. Journal of Physiology. 379:495–514.

Burges, R. A., A. J. Carter, D. F. Gardiner, and A. J. Higgins. 1985. Amlodipine, a new dihydropyridine calcium channel blocker with slow onset and long duration of action. British Journal of Pharmacology. 85:281P.

Burges, R. A., D. G. Gardiner, M. Gwilt, A. J. Higgins, K. J. Blackburn, S. F. Campbell, P. E. Cross, and J. K. Stubbs. 1987. Calcium channel blocking properties of amlodipine in vascular smooth muscle and cardiac muscle In Vitro: evidence for voltage modulation of vascular dihydropyridine receptors. Journal of Cardiovascular Pharmacology. 9:110-119.

Carbone, E., and H. D. Lux. 1988a. ω-Conotoxin blockade distinguishes Ca from Na permeable states in neuronal calcium channels. Pfliigers Archive 413:14–22.

Carbone, E., and H. D. Lux, 1988b. Sodium currents through neuronal calcium channels: kinetics and sensitivity to calcium antagonists. In The Calcium Channel: Structure, Function, and Implications. M. Morad, W. Nayler, S. Kazda, and M. Schramm, editors. Springer-Verlag. Heidelberg. 115–126.

Carbone, E., and H. D. Lux. 1989. Modulation of Ca channels in peripheral neurons. Annals of the New York Academy of Sciences. 560:346–357.

Catterall, William A. 1988. Structure and function of voltage-sensitive ion channels. Science. 242:50–60.

Flockerzi, V., H. J. Oeken, F. Hofmann, D. Pelzer, A. Cavalie, and W. Trautwein. 1986. Purified dihydropyridine-binding site from skeletal muscle T-tubules is a functional calcium channel. Nature. 325:66–68.

Gallozzi, J. P., M. Borsotto, J. Barhanin, M. Fosset, and M. Lazdunski. 1986. Characterization and photoaffinity labeling of receptor sites for Ca channel inhibitors d-cis-Diltiazem, Bepridil, Desmethoxyverapamil, and (+)-PN 200-110 in skeletal muscle transverse tubule membranes. Journal of Biological Chemistry. 144:211–215.

Glossmann, H., D. R. Ferry, A. Goll, J. Striessnig, and G. Zernig. 1984. Calcium channels: introduction into their molecular pharmacology. In Cardiovascular Effects of Dihydropyridine-Type Calcium Antagonists and Agonists. A. Fleckenstein, C. Van Breemen, R. Gross, and F. Hoffmeister, editors. Springer-Verlag. Heidelberg. 113–139.

Hadley, R. W., and J. R. Hume. 1987. An intrinsic potential-dependent inactivation mechanism associated with calcium channels in guinea-pig myocytes. Journal of Physiology. 399:205–222.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfliigers Archiv. 391:85–100.

Hamilton, S. L., A. Yatani, K. Brush, A. Schwartz, and A. M. Brown. 1987. A comparison between the binding and electrophysiological effects of dihydropyridines on cardiac membranes. Molecular Pharmacology. 31:221–231.

Hescheler, J., Pelzer, D., Trube, G., and W. Trautwein. 1982. Does the organic calcium channel blocker D600 act from inside or outside on the cardiac cell membrane? Pfliigers Archiv. 393:287–291.

Hess, P., J. B. Lansman, and R. W. Tsien. 1984. Different modes of gating behaviour favoured by dihydropyridine agonists and antagonists. Nature. 311:538–544.

Hess, P., J. B. Lansman, and R. W. Tsien. 1986. Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. Journal of General Physiology. 88:293–319.
Hille, B. 1977. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *Journal of General Physiology*. 69:497–515.

Hosey, M. H., and M. Lazdunski. 1988. Calcium channels: molecular pharmacology, structure and regulation. *Journal of Membrane Biology*. 104:81–105.

Iijima, T., T. Yanagisawa, and N. Taira. 1984. Increase in the slow inward current by intracellularly applied nifedipine and nicardipine in single ventricular cells of the guinea-pig heart. *Journal of Molecular and Cellular Cardiology*. 16:1173–1177.

Janis, R. A., and D. J. Triggle. 1991. Drugs acting on calcium channels. In *The Calcium Channel: Its Properties, Function, Regulation and Clinical Relevance*. L. Hurwitz, L. D. Partridge, and J. K. Leach, editors. CRC Press, Boca Raton, FL.

Kass, R. S. 1982. Nisoldipine: a new, more selective calcium current blocker in cardiac Purkinje fibers. *Journal of Pharmacology and Experimental Therapeutics*. 223:446–456.

Kass, R. S. 1987. Voltage-dependent modulation of cardiac calcium channel current by optical isomers of Bay K8644: implications for channel gating. *Circulation Research Supplement*. 61:11–115.

Kass, R. S., and J. P. Arena. 1989. Influence of pH on calcium channel block by amlopidine, a charged dihydropyridine compound: implications for location of the dihydropyridine receptor. *Journal of General Physiology*. 93:1109–1127.

Kass, R. S., and D. S. Krafte. 1987. Negative surface charge density near heart calcium channels. Relevance to block by dihydropyridines. *Journal of General Physiology*. 89:629–644.

Kokubun, S., B. Prod'hom, C. Becker, H. Forzig, and H. Reuter. 1986. Studies on Ca channels in intact cardiac cells: voltage-dependent effects and cooperative interactions of dihydropyridine enantiomers. *Molecular Pharmacology*. 30:751–584.

Leblanc N., and J. R. Hume. 1989. D600 block of L-type Ca$^{2+}$ channel in vascular smooth muscle cells: comparison with permanently charged derivative, D890. *American Journal of Physiology*. 257:C689–C695.

Lee, K. S., and R. W. Tsien. 1983. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialyzed heart cells. *Nature*. 302:790–794.

Lee, K. S., and R. W. Tsien. 1984. High selectivity of Ca channels in single dialyzed heart cells of the guinea pig. *Journal of Physiology*. 354:253–272.

Marchetti, C., and A. M. Brown. 1988. Protein kinase activator 1-oleoyl-2-acetyl-sn-glycerol inhibits two types of calcium currents in GH3 cells. *American Journal of Physiology (Cell)*. 250:C206–C210.

Mikami, A., K. Imoto, T. Tanabe, T. Niodome, Y. Mori, H. Takeshima, S. Narumiya, and Shosaku Numa. 1989. Primary Structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature*. 340:230–233.

Mitra, R., and M. Morad. 1985. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *American Journal of Physiology*. 249:H1056–H1060.

Nilius, B., P. Hess, J. B. Lansman, and R. W. Tsien. 1985. A novel type of cardiac calcium channel in ventricular cells. *Nature*. 316:443–446.

Perez-Reyes, E., H. S. Kim, A. E. Lacocqeda, W. Horne, X. Wei, D. Rampe, K. P. Campbell, A. M. Brown, and L. Birnbaumer. 1989. Induction of calcium currents by the expression of the $\alpha$-subunit of the dihydropyridine receptor from skeletal muscle. *Nature*. 340:233–236.

Prod'hom, B., D. Pietrobon, and P. Hess. 1989. Interactions of protons with single open L-type calcium channels. Location of protonation site and dependence of proton-induced current fluctuations on concentration and species of permeant ion. *Journal of General Physiology*. 94:23–42.

Pusch, M., and E. Neher. 1988. Rates of diffusional exchange between small cells and a measuring patch pipette. *Pflügers Archiv*. 411:204–211.
Sanguinetti, M. C., and R. S. Kass. 1984. Voltage-dependent block of calcium channel current in the calf cardiac Purkinje fiber by dihydropyridine calcium channel antagonists. Circulation Research. 55:336-348.

Sanguinetti, M. C., D. S. Krafte, and R. R. Kass. 1986. Bay K8644: voltage-dependent modulation of Ca channel current in heart cells. Journal of General Physiology. 88:369-392.

Sharp, A. H., T. Imagawa, A. T. Leung, and K. P. Campbell. 1987. Identification and characterization of dihydropyridine-binding subunit of the skeletal muscle dihydropyridine receptor. Journal of Biological Chemistry. 262:12309-12315.

Sieber, M., W. Nastainczyk, V. Zubor, W. Wernet, and F. Hormann. 1987. The 165-KDa peptide of the purified skeletal muscle dihydropyridine receptor contains the known regulatory sites of the calcium channel. European Journal of Biochemistry. 176:117-122.

Tanabe, T., K. Beam, J. A. Powell, and S. Numa. 1988. Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. Nature. 336:134-139.

Tanabe, T., H. Takeshima, A. Mikami, V. Flockerzi, H. Takahashi, K. Kangawa, M. Kojima, H. Matsuo, T. Hiose, and S. Numa. 1987. Primary structure of the receptor for calcium channel blockers from skeletal muscle. Nature. 328:313-318.

Tsien, R. W., P. Hess, E. W. McCleskey, and R. L. Rosenberg. 1987. Calcium channels: mechanisms of selectivity, permeation, and block. Annual Review of Biophysics and Biophysical Chemistry. 16:265-290.

Valdivia, H., and R. Coronado. 1990. Internal and external effects of dihydropyridines in the calcium channel of skeletal muscle. Journal of General Physiology. 95:1-27.

Wei, X. Y., E. M. Luchowski, A. Rutledge, C. M. Su, and D. M. Triggle. 1986. Pharmacological and radioligand binding analysis of the actions of 1,4-dihydropyridine activator-antagonist pairs in smooth muscle. Journal of Pharmacology and Experimental Therapeutics. 239:144-153.

Williams, J. S., I. L. Grupp, G. Grupp, P. L. Vagby, L. Dumont, A. Schwartz, A. Yatani, S. Hamilton, and A. M. Brown. 1985. Profile of the oppositely acting enantiomers of the dihydropyridine 202-791 in cardiac preparations: receptor binding, electrophysiological and pharmacological studies. Biochemical and Biophysical Research Communications. 131:13-21.