MOLECULAR PHARMACOLOGY, 27:544–548

Conformational Features of Calcium Channel Agonist and Antagonist Analogs of Nifedipine

DAVID A. LANGS AND DAVID J. TRIGGLE

Department of Molecular Biophysics, Medical Foundation of Buffalo, Inc., Buffalo, New York 14203 and Department of Biochemical Pharmacology, State University of New York at Buffalo, Buffalo, New York 14260

Received December 6, 1984; Accepted February 13, 1985

SUMMARY

The crystal and molecular structures of methyl 2,6-dimethyl-5-nitro-4-(2-trifluoromethylphenyl)-1,4-dihydropyridine-3-carboxylate and ethyl 4-(2-difluoromethoxyphenyl)-1,4,5,7,-tetrahydro-2-methyl-5-oxofuro[3,4-*b*]pyridine-3-carboxylate, which are analogs of the calcium channel antagonist nifedipine reported to have agonist activity, have been determined. The conformations of these two agonists are compared with the conformational features shown by nifedipine and related 1,4-dihydropyridine calcium channel antagonists. Common conformational features shown by these agonists and antagonists allow both to bind to the same plasma membrane receptor while subtle differences in hydrogen-bonding activity of the amine group, and ester group orientation and hydrophobic fit, may control the availability of channel open and closed states.

INTRODUCTION

Nifedipine (*I*, Fig. 1) and related DHP[1] calcium channel antagonists are important cardiovascular drugs which inhibit smooth and cardiac muscle contraction by blocking the influx of calcium ions through plasma membrane channels (1–3). All 1,4-dihydropyridine antagonists appear to act at a common DHP membrane-binding site and show competitive inhibition of binding of various radiolabeled analogs such as [^{3}H]nitrendipine (*II*) (4–9). Correlations between pharmacologic and membrane-binding activities establish that these binding sites are pharmacologically relevant (2, 8, 9). These drugs do not bind specifically to other well known membrane receptors (2, 8) which have been characterized for neurotransmitters, modulators, and hormones. Furthermore, other well known non-DHP calcium channel antagonists such as verapamil and diltiazem act at cell membrane-binding sites, which are structurally distinct from, but linked to, the DHP receptor (5–11).

Methyl 2,6-dimethyl-5-nitro-4-(2-trifluoromethylphenyl)1,4-dihydropyridine-3-carboxylate (*III*, BAY K 8644) is a potent analog of nifedipine which produces positive inotropic and pressor responses in tissues of the cardiovascular system (12–15). These effects are produced at concentrations comparable to those required of nifedipine and its related equipotent 2-trifluoromethylphenyl analog (*IV*) (16–18) to produce cardiac and smooth muscle relaxation. Competitive interactions between (*III*) and DHP antagonists, seen in pharmacologic and radioligand-binding assays, indicate that they bind to the same DHP calcium channel receptor (14, 15, 19). Ethyl 4-(2-difluoromethoxyphenyl)-1,4,5,7-tetrahydro-2-methyl-5-oxofuro[3,4-*b*]pyridine-3-carboxylate (*V*, CGP 28 392) has been also reported to possess agonist activity similar to *III*, although it is somewhat less potent (15, 20). Thus, 1,4-dihydropyridines that are closely related in structure can generate agonist or antagonist activities at the calcium channel. Recent direct measurements of calcium channel currents in single cells (21, 22) have indicated that agonist binding promotes an open state of the receptor-channel complex while antagonist binding increases the probability that the channel will remain closed. Diffraction analyses of *III* and *V* were undertaken in an attempt to identify those molecular characteristics which permit these two agonists and related antagonists to bind to the same DHP receptor and yet evoke opposite pharmacologic responses.

MATERIALS AND METHODS

Crystals of *III* obtained from the evaporation of ethyl acetate are monoclinic $P2_1/c$ with $a = 10.769(2)$, $b = 12.762(2)$, $c = 12.603(2)$ Å, $\beta = 108.61(2)°$, $Z = 4$, volume = 1641 Å^3. A total of 4394 intensity data were measured with MoKα radiation ($\lambda = 0.7107$ Å) at 90° K in the automated θ–2θ scan mode on a Syntex/Nicolet P3 four-circle diffractometer; of these, 4059 were observed with $|F| > 2\sigma(F)$. The crystal structure was determined by multisolution procedures (23) and refined by full matrix least squares methods. Hydrogen coordinates were refined with isotropic temperature factors; non-hydrogen atoms were assigned anisotropic thermal values in the refinement. The final residuals were $R = 0.064$ and $R_w = 0.072$. The average estimated standard deviations of the non-hydrogen bond lengths and angles are ±0.0025 Å and ±0.1°. The range, mean, and computed standard deviation of the 14 refined C-H bonds are 0.87–1.02 and 0.95 ± 0.04 Å, which compares

This work was supported by United States Public Health Service Grants HL32303, T32 AM07368 (D. A. L.), and HL16003 (D. J. T.).

[1] The abbreviation used is: DHP, dihydropyridine.

0026-895X/85/050544–05$02.00/0

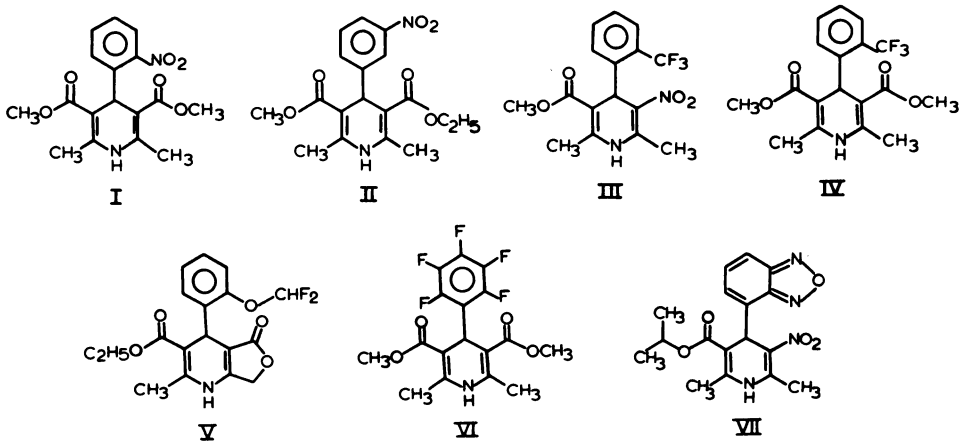

FIG. 1. *Molecular formulae of the various nifedipine analog compounds*

Compounds *I*, *II*, *IV*, and *VI* are potent antagonists while compounds *III* and *V* are agonist dihydropyridines. The *R* enantiomer of compound *VII* possesses antagonist activity as opposed to the *S* enantiomer which exhibits agonist activity.

well with the estimated standard deviation of ±0.03 Å derived from the full matrix least squares refinement.

Compound *V* was crystallized from acetone as monoclinic diamond-shaped plates: $P2_1/c$, $a = 11.436(3)$, $b = 10.941(2)$, $c = 14.361(4)$ Å, $\beta = 108.40(3)°$, $Z = 4$, volume = 1705 Å^3. A total of 3541 data was measured at room temperature with CuKα radiation ($\lambda = 1.5418$ Å) on a Nonius CAD-4 diffractometer; of these, 2103 were observed with $|F| > 2\sigma(F)$. The final least squares residuals were $R = 0.080$ and $R_w = 0.087$, and the average estimated standard deviations of the non-hydrogen bond lengths and angles are ±0.006 Å and ±0.3°. The range, mean, and computed standard deviation of the 16 C-H bonds are 0.85–1.09 and 0.97 ± 0.07 Å, compared with the estimated standard deviation of ± 0.06 Å determined from the least squares refinement.

RESULTS

The molecular structures[2] of *III* and *V* shown[3] in Figs. 2 and 3 clearly depict the flatness of the expected boat conformation of the DHP ring and the restriction of aryl ring rotation to the DHP vertical N1-C4 symmetry plane for both of these compounds. The restricted aryl ring orientation of *III* is largely due to overcrowding contacts between the CF_3 fluorine atoms and the carbonyl ester and nitro oxygen atoms which are directed toward the H4 bowsprit side of the DHP ring. Prior examination of molecular models of *III* suggested that the CF_3 group should adopt a staggered orientation to position adjacent fluorines on either side of the C4 hydrogen atom, and cause either or both the ester and nitro groups to rotate significantly from their normal plane of attachment to the DHP ring. This staggered orientation would avoid an untenable H4-F contact of less than 1.4 Å which would result if the CF_3 group were rotated to eclipse one of the fluorine atoms with the H4 position. However, the CF_3 group was found to be in the eclipsed orientation (Fig. 2). The model bond angles at C7, C12, and C13 each increased by more than 6° to extend the H4-F contact to 2.2 Å. The severity of the fluorine contacts with the bowsprit carbonyl ester and nitro oxygen atoms is greatly reduced when CF_3 is eclipsed, and although the aromatic ring is virtually locked to within 6° of the vertical DHP symmetry plane, these contacts are not sufficient to force the ester or nitro groups to rotate out of the plane of the DHP ring.

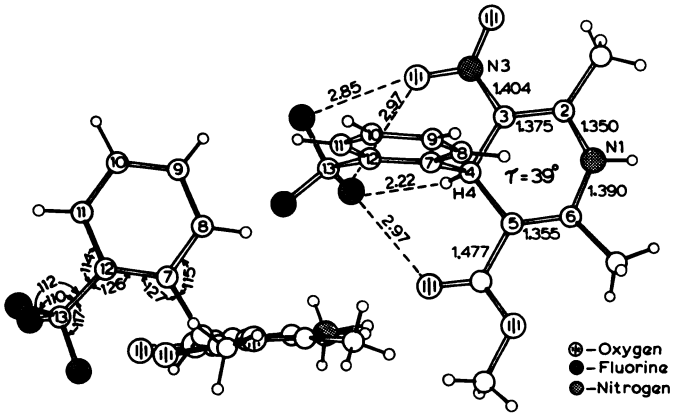

FIG. 2. *Orthogonal perspective views of the molecular conformation of compound III*

Enlarged bond angles and short fluorine contact distances characterize the intramolecular steric overcrowding of the CF_3 group.

The DHP rings of *III* and *V* were both found to be flatter than had previously been noted for nifedipine and other potent DHP antagonists (24, 25). The sum of the magnitudes of the DHP ring torsion angles of *III* and *V* are only 39° and 47°, respectively, as compared to the minimum value of 52° observed for the very potent antagonist methyl 2,6-dimethyl-4(pentafluorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (*VI*) (25). The flatness and 5-nitro substitution of the DHP ring of molecule *III* appear to permit a delocalization of electrons from the amine toward the nitro group. This observation is supported by perturbations of indicated bond lengths further illustrated by canonical forms in Fig. 4. The N1-C2 and C3-N3 bond lengths are significantly shorter and the C2-C3 bond longer [1.350(3), 1.404(3), 1.375(2) Å] than their normal respective average values [1.380(6), 1.471(4), 1.359(7) Å] seen in the seven most

[2] The fractional atomic coordinates for compounds *III* and *V* have been deposited in the Cambridge Crystallographic Database. Copies may be obtained from the Cambridge Crystallographic Data Centre, Cambridge, England CB2 1EW by citing this journal article.

[3] Composed on the National Institutes of Health PROPHET computer system.

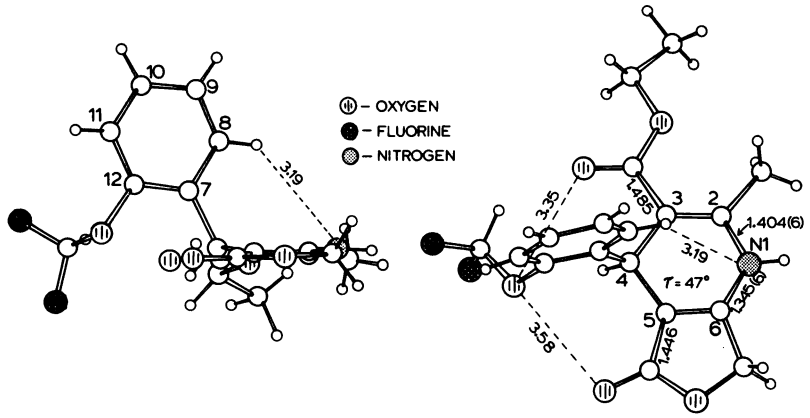

FIG. 3. *Perspective views of the molecular conformation of compound V*

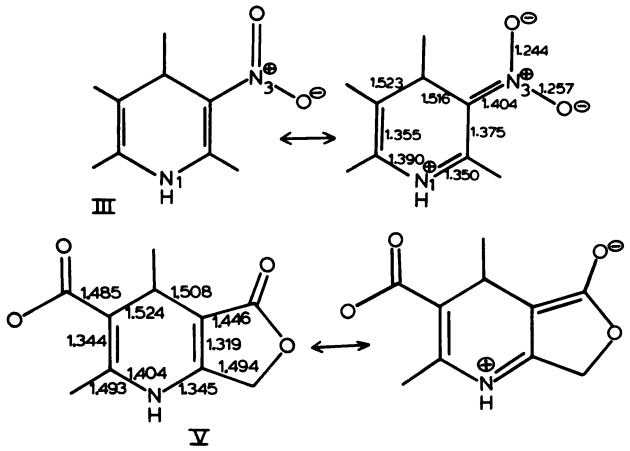

FIG. 4. *Chemical bond lengths and regions of molecular bond planarity suggest that the dihydropyridine double bonds of compound III have been delocalized as is indicated by the canonical form on the right*

Similar bond length perturbations in compound *V* may indicate an analogous resonance situation as is depicted.

accurately refined nifedipine analog crystal structures (24, 25). Such charge delocalization may be anticipated in structures in which a nitro group substitutes directly adjacent to a localized double bond, as has been previously observed in a comparison of the crystal structures of uracil and 5-nitrouracil (26). Given that the plane of the nitro group of *III* is within 2.8° of the plane of the C2-C3 bond, these data suggest that the amine group of *III* is significantly more acidic and capable of forming stronger hydrogen donor bonds than the undelocalized amine functions of DHP antagonists. The crystal structure of compound *III* discloses that the amine group of the molecule is found to have the potential to enter into any one of three geometrically favorable hydrogen-bonding arrangements, depending on whether the amine is sp^2 hybridized, as observed, or sp^3 hybridized with the hydrogen either above or below the DHP plane. Compound *V* is found to crystallize with the amine nitrogen forming a single hydrogen bond. An examination of the crystal structures of the 15 previously analyzed DHP antagonists (24, 25) reveals that the amine functions of these molecules always form a single hydrogen bond in those crystal structures.

DISCUSSION

Quantitative structure-activity relationships have been derived for nifedipine analogs (17) in which antagonist potency correlates with large values of the minimum width Verloop (27) Sterimol parameter, B_1, of the *ortho*- or *meta*-aryl substituent and the lipophilic and steric factors of the ester group substituent. The correlation with the Verloop parameter supports an earlier hypothesis (16) that bulky *ortho* substituents restrict the rotational orientation of the aryl ring to be perpendicular to the DHP ring, with the *ortho* substituent sandwiched between the ester oxygen atoms which flank the bowsprit end of the molecule. Subsequent diffraction studies (24, 25) confirmed this hypothesis and showed that the DHP ring assumed a flattened boat conformation with the phenyl ring positioned above the boat in a "flagpole" (axial) conformation and the aryl substituent preferentially positioned on the outside "bowsprit" side of the molecule; *meta*-substituted antagonists were found to possess this same overall conformation to indicate that dipole effects rather than steric factors influence the same aryl ring orientation above the DHP ring. The diffraction studies revealed a stronger correlation between DHP ring flatness and antagonist activity than was found in terms of the degree of aryl ring rotational restriction to the N1-C4 vertical plane of the DHP ring. The more active antagonists have aryl ring substituents which tend to both restrict the aromatic ring to the DHP vertical plane and flatten the DHP ring. That these conformational requirements are shared by the agonists reported here is consistent with the thesis that agonists and antagonists interact at the same site.

The role of the ester groups in the activity profile of the 1,4-DHP ligands requires further clarification. Quantitative structure-activity relationships studies underscore the importance of lipophilicity and steric factors of the ester group (17) to conform to hydrophobic pockets in the DHP receptor. The observed tissue selectivities (28–33) and chiral preferences (32–35) of certain dissymmetric ester analogs call attention to differences in the sizes and dispositions of these hydrophobic pockets between different tissues. It has yet to be established to what extent this selectivity may be based on chiral hand-

edness imposed by differences between *cis* and *trans* orientations of the ester groups.

The majority of the antagonist analogs of nifedipine (13 of 16) are found to have both a *cis* and *trans* ester group orientation whereby the carbonyl bond of one ester function is oriented *cis* to one of the DHP ring double bonds, and the carbonyl bond of the second ester group is directed *trans* relative to the second ring double bond. The *cis* ester positions the ester group forward to the bowsprit (C4) side of the DHP ring adjacent to the face of the aryl ring while a *trans* ester places the ester group far to the "port" or "starboard" sides of the DHP boat. A smaller number of these antagonists (3 of 16), which represent three of the most active analogs, show a *cis-cis* ester geometry. No antagonist compounds are known to crystallize with a *trans-trans* ester arrangement. The significance of ester orientation to pharmacologic activity remains to be determined. However, the fact that conformationally constrained nifedipine analogs which have a *cis* ester group covalently linked to the distal *ortho* side of the phenyl ring are active antagonists, provided that the connecting chain is long enough to permit the phenyl group to position itself near the N1-C4 vertical plane of the molecule (36), indicates that at least one *cis* ester group is tolerated for antagonist activity. Similar studies utilizing *trans*-restricted analogs have yet to be devised to ascertain whether specific ester conformations offer necessary or sufficient conditions for calcium channel antagonism. That compounds *III* and *V* are the first pharmacologically active nifedipine analogs which lack a potential for two *cis* ester groups suggests that the question of ester group conformation should be more thoroughly investigated to conclude whether it is conformation or the lack of an ester group substituent which is crucial for the determination of agonist versus antagonist activity.

Receptor binding of both activator and antagonist analogs of nifedipine requires aryl ring substituents which restrict that ring to the DHP vertical plane and subsequently flatten the DHP boat conformation. The differentiation of agonist versus antagonist response by the receptor may be dictated by differences in the hydrogen-bonding strength of the amine group and discrimination between the bonding loci and orientations of the ester groups. Asymmetric occupancy of binding sites for the 3- and 5-substituents of the 1,4-DHP ring is likely to be an important determinant for agonist/antagonist activity because the *R* and *S* enantiomers of isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitropyridine-3-carboxylate (*VII*) exhibit antagonist and activator properties, respectively (37). The opposing activities of these enantiomers suggest that differences in hydrogen-bonding strengths of the amine groups are not required to differentiate between agonist and antagonist responses.

ACKNOWLEDGMENTS

We thank Dr. A. Scriabine of the Miles Institute of Preclinical Pharmacology, New Haven, CT and Dr. G. B. Weiss of Ciba-Geigy, Summit, NJ for providing the original samples of *III* and *V*, respectively. We appreciate the receipt of a prepublication copy of Ref. 37 from Prof. R. P. Hof.

REFERENCES

1. Fleckenstein, A. Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.* **17**:149–166 (1977).
2. Janis, R. A., and D. J. Triggle. New developments in Ca^{2+} channel antagonists. *J. Med. Chem.* **26**:775–785 (1983).
3. Lee, K. S., and R. W. Tsien. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature* **302**:790–794 (1983).
4. Bellemann, P., D. Ferry, F. Lubbecke, and H. Glossmann. [^{3}H]Nitrendipine, a potent calcium antagonist, binds with high affinity to cardiac membranes. *Arzneim. Forsch. Drug Res.* **31**:2064–2067 (1981).
5. Gould, R. J., K. M. M. Murphy, and S. H. Snyder. [^{3}H]Nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc. Natl. Acad. Sci. USA* **79**:3656–3660 (1982).
6. Ehlert, F. J., E. Itoga, W. R. Roeske, and H. I. Yamamura. The binding of [^{3}H]nitrendipine to receptors for calcium channel antagonists in the heart. *Life Sci.* **30**:2191–2202 (1982).
7. Glossmann, H., D. R. Ferry, F. Lubbecke, R. Mewes, and F. Hofmann. Calcium channels: direct identification with radioligand binding studies. *Trends Pharmacol. Sci.* **3**:431–437 (1982).
8. Bolger, G. T., P. Genco, R. Klockowski, E. Luchowski, H. Siegel, R. A. Janis, A. M. Triggle, and D. J. Triggle. Characterization of binding of the Ca^{2+} channel antagonist, [^{3}H]nitrendipine, to guinea-pig ileal smooth muscle. *J. Pharmacol. Exp. Ther.* **225**:291–309 (1983).
9. Bellemann, P., A. Schade, and R. Towart. Dihydropyridine receptor in rat brain labeled with [^{3}H]nimodipine. *Proc. Natl. Acad. Sci. USA* **80**:2356–2360 (1983).
10. Yamamura, H. I., H. Schoemaker, R. G. Boles, and R. W. Roeske. Diltiazem enhancement of [^{3}H]nitrendipine binding to calcium channel associated drug receptor sites in rat brain synaptosomes. *Biochem. Biophys. Res. Commun.* **108**:640–646 (1982).
11. Murphy, K. M. M., R. J. Gould, B. L. Largent, and S. H. Synder. A unitary mechanism of calcium antagonist drug action. *Proc. Natl. Acad. Sci. USA* **80**:860–864 (1983).
12. Schramm, M., G. Thomas, R. Towart, and G. Franckowiak. Novel dihydropyridine with positive inotropic action through activation of Ca^{2+} channels. *Nature* **303**:535–537 (1983).
13. Schramm, M., G. Thomas, R. Towart, and G. Franckowiak. Activation of calcium channels by novel 1,4-dihydropyridines: a new mechanism for positive inotropics on smooth muscle stimulants. *Arzneim. Forsch. Drug Res.* **33**:1268–1272 (1983).
14. Su, C. M., R. A. Janis, and D. J. Triggle. Bay k 8644, a calcium channel activator in smooth muscle. *Fed. Proc.* **43**:150 (Abstr. 1551) (1984).
15. Su, C. M., V. C. Swamy, and D. J. Triggle. Calcium channel activation in vascular smooth muscle by Bay k 8644. *Can. J. Physiol. Pharmacol.* **62**:1401–1413 (1984).
16. Loev, B., M. M. Goodman, K. M. Snader, R. Tedeschi, and E. Macko. "Hantzch-type" dihydropyridine hypotensive agents. *J. Med. Chem.* **17**:956–965 (1974).
17. Rodenkirchen, R., R. Bayer, R. Steiner, E. Bossert, H. Meyer, and E. Moller. Structure-activity studies on nifedipine in isolated cardiac muscle. *Naunyn-Schmiedebergs Arch. Pharmacol.* **310**:69–78 (1979).
18. Triggle, D. J., and V. C. Swamy. Calcium antagonists: some chemical-pharmacologic aspects. *Circ. Res.* **52**:(Suppl. I):17–28 (1982).
19. Janis, R. A., D. Rampe, J. G. Sarmiento, and D. J. Triggle. Specific binding of a calcium channel activator, [^{3}H]Bay k 8644, to membranes from cardiac muscle and brain. *Biochem. Biophys. Res. Commun.* **121**:317–323 (1984).
20. Truog, A. G. Presentation at the Federation of American Societies for Experimental Biology, 67th Annual Meeting, Chicago, April 1983.
21. Brown, A. M., D. L. Kunze, and A. Yatani. The agonist effect of dihydropyridines on Ca channels. *Nature* **311**:570–572 (1984).
22. Hess, P., J. B. Lansman, and R. W. Tsien. Different modes of Ca channel gating behavior favoured by dihydropyridine Ca agonists and antagonists. *Nature* **311**:538–544 (1984).
23. Main, P., L. Lessinger, M. M. Woolfson, G. Germain, and J. P. Declerq. *MULTAN 77: a System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Universities of York, England and Louvain, Belgium (1977).
24. Fossheim, R., K. Swarteng, A. Mostad, C. Romming, E. Shefter, and D. J. Triggle. Crystal structures and pharmacological activity of calcium channel antagonists 2,6-dimethyl-3,5-dicarbomethoxy-4-(unsubstituted, 3-methyl-, 4-methyl-, 3-nitro-, 4-nitro-, and 2,4-dinitrophenyl)-1,4-dihydropyridine. *J. Med. Chem.* **25**:126–131 (1982).
25. Triggle, A. M., E. Shefter, and D. J. Triggle. Crystal structures of calcium channel antagonists: 2,6-dimethyl-3,5-dicarbomethoxy-4-[2-nitro-, 3-cyano-, 4-(dimethylamino)-, and 2,3,4,5,6-pentafluorophenyl]-1,4-dihydropyridine. *J. Med. Chem.* **23**:1442–1445 (1980).
26. Craven, B. M. The crystal structure of 5-nitrouracil monohydrate. *Acta Crystallogr.* **23**:376–383 (1967).
27. Verloop, A., W. Hoogstraaten, and J. Tipker. Development and application of new steric substituent parameters in drug design, in *Drug Design* (E. J. Ariens, ed.), Vol. 7. Academic Press, New York, 165–207 (1976).
28. Kazda, S., B. Garthoff, and A. Knorr. Nitrendipine and other calcium entry

blockers (calcium antagonists) in hypertension. *Fed. Proc.* **42**:196–220 (1983).
29. Hashimoto, K., K. Takeda, Y. Katano, Y. Nakagowa, T. Tsukada, T. Hashimoto, N. Shimamoto, K. Sakai, T. Otorrii, and S. Imai. Effects of niludipine (Bay a 7168) on the cardiovascular system. *Arzneim. Forsch. Drug Res* **29**:1368–1373 (1979).
30. Kuwajima, I., K. Ueda, and M. Murakami. A study of the effects of niludipine in hypertensive patients. *Arzneim. Forsch Drug Res.* **32**:398–402 (1982).
31. Kazda, S., B. Garthoff, H. Meyer, K. Schlossmann, K. Stoepel, R. Towart, W. Vater, and E. Wehinger. Pharmacology of a new calcium antagonist compound isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine carboxylate (nisoldipine, Bay k 5552). *Arzneim. Forsch. Drug Res.* **30**:2144–2162 (1979).
32. Takenaka, T., I. Miyazaki, M. Asano, S. Higuchi, and H. Maeno. Vasodilator and hypotensive effects of the optical isomers of nicardipine (YC-93), a new calcium antagonist. *Jpn. J. Pharmacol.* **32**:665–670 (1983).
33. Towart, R., E. Wehinger, H. Meyer, and S. Kazda. The effects of nimodipine, its optical isomers and metabolites on isolated vascular smooth muscle. *Arzneim. Forsch. Drug Res.* **32**:338–346 (1982).
34. Towart, R., E. Wehinger, and H. Meyer. Effects of unsymmetrical ester substituted 1,4-dihydropyridine derivatives and their optical isomers on contraction of smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmacol.* **317**:183–185 (1981).
35. Kazda, S., B. Garthoff, H. P. Krause, and K. Schlossmann. Cardiovascular effects of the calcium antagonistic dihydropyridine derivative nimodipine in animal experiments. *Arzneim. Forsch. Drug Res.* **32**:331–338 (1982).
36. Seidel, W., H. Meyer, L. Born, S. Kazda, and W. Dompert. Rigid calcium antagonists of the nifedipine type: geometrical requirements for the dihydropyridine receptor. Presented at the Division of Medical Chemists meeting, American Chemical Society, 187th National Meeting, St. Louis, MO, April 8–14 (Abstr. 14) (1984).
37. Hof, R. P., U. T. Ruegg, A. Hof, and A. Vogel. Stereoselectivity at the calcium channel: opposite action of the enantiomers of a 1,4-dihydropyridine. *J. Cardiovasc. Pharmacol.* **7**: in press (1985).

Send reprint requests to: David A. Langs, Department of Molecular Biophysics, Medical Foundation of Buffalo, Inc., 73 High Street, Buffalo, NY 14203.