

## Receptor model for the molecular basis of tissue selectivity of 1,4-dihydropyridine calcium channel drugs

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### SUMMARY

Our analysis of the solid state conformations of nifedipine [dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinecarboxylate] and its 1,4-dihydropyridine (1,4-DHP) analogues produced a cartoon description of the important interactions between these drugs and their voltage-dependent calcium channel receptor. In the present study a molecular-level detailed model of the 1,4-DHP receptor binding site has been built from the published amino acid sequence of the  $\alpha_1$  subunit of the voltage-dependent calcium channel isolated from rabbit skeletal muscle transverse tubule membranes. The voltage-sensing component of the channel described in this work differs from others reported for the homologous sodium channel in that it incorporates a water structure and a staggered, rather than eclipsed, hydrogen bonded S4 helix conformation. The major recognition surfaces of the receptor lie in helical grooves on the S4 or voltage-sensing  $\alpha$ -helix that is positioned in the center of the bundle of transmembrane helices that define each of the four calcium channel domains. Multiple binding clefts defined by Arg-X-X-Arg-P-X-X-S 'reading frames' exist on the S4 strand. The tissue selectivity of nifedipine and its analogues may arise, in part, from conservative changes in the amino acid residues at the P and S positions of the reading frame that define the ester-binding regions of receptors from different tissues. The crystal structures of two tissue-selective nifedipine analogues, nimodipine [isopropyl (2-methoxyethyl) 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylate] and nitrendipine [ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylate] are reported. Nimodipine was observed to have an unusual ester side chain conformation that enhances the fit to the proposed ester-sensing region of the receptor.

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### INTRODUCTION

Nifedipine and related 1,4-dihydropyridines (1,4-DHPs) are an important group of drugs that are potent blockers of calcium ( $\text{Ca}^{2+}$ ) currents through the L class of voltage-dependent  $\text{Ca}^{2+}$

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channels [for review see Ref. 1]. This activity underlies their role in cardiovascular medicine. Radioligand binding studies have used [<sup>3</sup>H] 1,4-DHPs to establish the existence of specific membrane binding sites that mediate the pharmacologic response to these agents. Nifedipine analogues with non-identical ester groups, unlike their symmetrical ester analogues, often exhibit tissue selectivities and chiral preferences of potential clinical significance. Among such reported tissue-selective compounds, nitrendipine lowers elevated peripheral vascular resistance [2] and is useful as an antihypertensive agent, while nimodipine is a cerebral vasodilator [3] which may be of value in the treatment of cerebrovascular vasospasm following stroke. Nimodipine may attract additional attention as an agent used to improve memory skills among aging individuals [4]. The crystal structures of these two compounds were determined to permit comparisons of their DHP ring deformations and ester group conformations, molecular features thought to be important in describing the structure-activity relationships of these agents.

## METHODS

### *Crystal structure determination*

Single crystals of nitrendipine and nimodipine suitable for X-ray diffraction were grown by evaporation from ethanol solutions at 276 K. Unit cell, data collection and structure refinement parameters are given in Table 1\*. The data were recorded by a stepped-scan procedure and analysed for statistical significance using the data processing programs developed by Blessing [5]. The crystal structures were determined by the direct methods program MULTAN [6] and refined by full-matrix, least-squares procedures.

### *Molecular modeling*

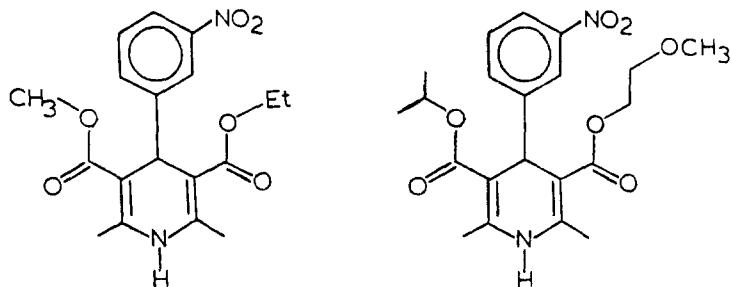
The SYBYL (Tripos Associates, Inc., St. Louis, MO) molecular modeling routines were used on an Evans & Sutherland PS390 graphics workstation. Putative transmembrane segments of the  $\alpha_1$  subunit of the rabbit skeletal muscle peptide sequence [7] were initially modeled as  $\alpha$ -helices with random side chain conformations employing the SYBYL BIOPOLYMER subroutine; crystallographic coordinates of nifedipine drug analogues were introduced through the CRYSTIN subroutine. Molecular docking exercises were performed using the TWIST routine to alter the amino acid side chain conformations and the MONITOR routine to actively display potential intermolecular hydrogen bonds and van der Waals contacts between nifedipine drugs and various tested polypeptide surfaces. The objective was to optimise the hydrogen bonding geometries with donor protons and lone pair orbitals directed toward one another at reasonable hydrogen bonding distances (2.75–3.0 Å), while also maximizing the fit of hydrophobic interactions by avoiding van der Waals contacts shorter than 3.25 Å. Full geometry energy minimizations were performed using the SYBYL program module MAXIMIN2 employing the TRIPPOS basis set of force field parameters.

## RESULTS AND DISCUSSION

Stereoviews of the molecular structures of nitrendipine and nimodipine are shown in Fig. 1. *Cis* (*sp*, synperiplanar) and *trans* (*ap*, antiperiplanar) ester groups are identified as having the carbon-

\*Atomic coordinates have been deposited with the Cambridge Structural Database, Cambridge Crystallographic Centre, Cambridge CB2 1EW, U.K.

TABLE I  
UNIT CELL, DATA COLLECTION AND STRUCTURE REFINEMENT PARAMETERS FOR NITRENDIPINE  
AND NIMODIPINE



	NITRENDIPINE	NIMODIPINE
	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub>	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>7</sub>
Temperature	130 K	295 K
Space Group	P2 <sub>1</sub> /c	P2 <sub>1</sub> /c
a =	8.812(2) Å	13.933(1) Å
b =	15.292(4)	10.985(1)
c =	12.956(3)	14.839(1)
$\beta$ =	93.66(2) $^\circ$	104.90(1) $^\circ$
V =	1742.2(13) Å <sup>3</sup>	2194.9(6) Å <sup>3</sup>
Z =	4	4
Diffractometer	Nicolet P3	Nonius CAD-4
X-Irradiation	Mo K $\alpha$	Cu K $\alpha$
(sin $\theta$ / $\lambda$ ) max	0.595	0.628
# I <sub>h</sub>	3096	4556
# I <sub>h</sub> > 2 $\sigma$ (I <sub>h</sub> )	2301	3938
R =	7.8%	6.7%
R <sub>w</sub> =	6.4%	9.9%
S =	1.49	2.14
< $\sigma$ (C-C)>	0.003 Å	0.003 Å
< $\sigma$ (C-C-C)>	0.2 $^\circ$	0.1 $^\circ$
< $\sigma$ (C-H)>	0.03 Å	0.04 Å
$\sigma$ <(C-H)>	0.09 Å	0.09 Å

yl double bonds either *cis* or *trans* to the neighboring double bond in the DHP ring. Nitrendipine crystallizes in an *sp,ap* ester conformation with the ethyl group in the *ap* orientation. Both ester groups of the nimodipine structure are found in *sp* conformations; the linear side chain of the methoxyethyl ester folds upwards and lies parallel to the planar face of the aryl ring as shown in Fig. 1B.

The molecular conformations of nitrendipine and nimodipine are very similar to those reported

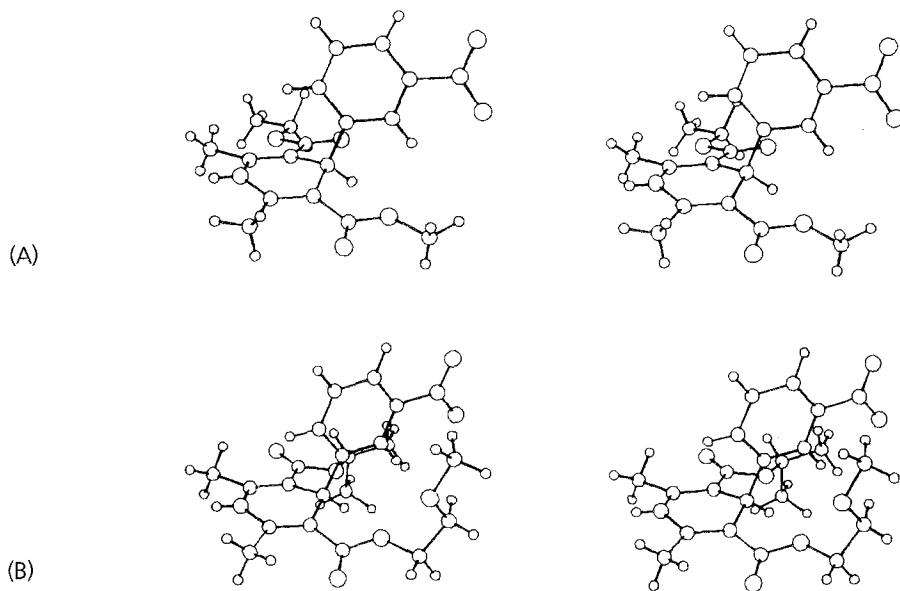


Fig. 1. Stereoscopic views showing the molecular conformations of (A) nitrendipine and (B) nimodipine. Note that the methoxyethyl side chain of nimodipine is seen to fold upwards, positioned parallel to the flat face of the aryl ring.

for numerous nifedipine analogues [8-16]. The 1,4-DHP ring adopts a boat conformation with the N(1) and C(4) atoms defining the stern and bow positions. The aryl ring is positioned above the concave side of the DHP ring in an axial or flagpole orientation with the plane of the phenyl ring aligned approximately coplanar with the N(1)-C(4) vertical plane of the DHP ring. The aryl ring substituent is almost always found on the outer or bowsprit side of the phenyl ring. The carboxyl portions of the ester groups are essentially coplanar with the adjoining C-C bonds of the DHP ring to which they are attached. Among the 27 known or reported crystal structures of nifedipine antagonists, 18 molecules are found to have *sp,ap* ester conformations, the remaining 9 are *sp,sp*, and no example of an *ap,ap* ester conformation has yet been observed although it is generally believed that the energy differences between such ester conformations are not large.

#### *Structure-activity studies*

Early structure-activity analyses of nifedipine analogues bearing symmetric ester groups noted certain stereochemical factors that constrain the phenyl ring to lie close to the N(1)-C(4) vertical bisecting plane of the DHP ring [17]. Diffraction studies confirmed this hypothesis and also disclosed a stronger structure-activity relationship that correlated antagonist activity with the degree of planarity of the boat conformation of the DHP ring [8,15]. In these analyses the degree of puckering was described as a simple sum of the absolute magnitudes of the six torsion angles of the DHP ring. This variable,  $\Sigma|\rho|$  ranged from a low of 52 and 59 degrees for two observations of the most active antagonist to a high of 121 degrees for one of the least active analogues. Corresponding values of  $\Sigma|\rho|$  for the nimodipine and nitrendipine structures are 82 and 97 degrees, respectively; both are within eight degrees of the 90-degree value reported for the related 3-nitrophenyl dimethyl ester analogue [8].

QSAR studies of various nifedipine analogues in cardiac muscle by Rodenkirchen et al. [18,19] noted that the lipophilic and bulk steric properties of the ester groups and large values of the minimum width Verloop sterimol parameter  $B_1$  of the 4-phenyl ring substituent were important factors for enhancing antagonist potency and receptor binding. Large values of this  $B_1$  parameter for *ortho*-phenyl analogues would ensure that the *ortho* substituent was spatially ‘sandwiched’ between the pair of ester groups on the outer bowsprit side of the molecule. This constrains the aryl ring to lie coplanar with the N(1)-C(4) vertical plane of the DHP ring. In a series of conformationally constrained nifedipine analogues in which a chemical bridge was introduced between the phenyl and 1,4-DHP rings [20,21], it was noted that activity was highest for those analogues where the phenyl ring lies closest to the vertical plane of the DHP ring.

QSAR methods have subsequently been extended to a much larger group of compounds to describe their activity in smooth muscle [22,23]. The method of partial least squares analysis of latent variables [24] has been applied in an attempt to improve the predictive QSAR between the published crystallographic molecular torsion angles and physiochemical parameters of selected nifedipine analogues and their  $\text{Ca}^{2+}$  channel antagonist activity as estimated by  $\log ID_{50}$ . Molecular graphics techniques have recently been used by Höltje and Marrer [25] to propose a hypothetical receptor/drug binding model based on correlations between the biological activities ( $-\log IC_{50}$ ,  $\text{K}^+$ -stimulated contraction of rabbit aorta) and calculated interaction energies of various tested models. A high correlation ( $r=0.94$ ,  $s=0.28$ ,  $n=7$ ) was found for one binding model in which nifedipine antagonist molecules experienced three sites of interaction with certain amino acid side chains: (i) the indole ring of Trp with an alkyl group of a port side, *cis*-directed ester, (ii) the charged amine of Lys with an *ortho*- or *meta*-nitrophenyl substituent in the bowsprit *sp* ring orientation, (iii) the aromatic ring of Phe or Trp with an alkyl group of a starboard side, *trans*-directed ester.

#### *Characteristics of the 1,4-DHP receptor*

In a recent review [1], attention was directed to a number of conformational characteristics which could have a direct bearing on the modes of ligand-receptor binding to the various gating states of the  $\text{Ca}^{2+}$  L-type channel. It was noted that the degree of ring pucker markedly influences the angle of tilt of the aryl ring as sensed by the direction of its flagpole. If one quantifies this angle of tilt by  $\Sigma |\bar{\tau}|$ , the average magnitude of the C(2)-C(3)-C(4)-C(7) and C(6)-C(5)-C(4)-C(7) torsion angles, the linear regression between  $\Sigma |\rho|$  and  $\Sigma |\bar{\tau}|$  for 33 crystallographically determined nifedipine analogues, both antagonists and activators, has a correlation coefficient of 0.97 and an average [rms] displacement of only 1.4 degrees. Thus these two modes of conformational adjustment are not independent of one another, and either parameter can be used as a measure of the joint overall conformation of DHP and aryl rings. In addition, as is indicated by the 3-nitrophenyl analogues in this study, the values of  $\Sigma |\rho|$  and  $\Sigma |\bar{\tau}|$  are largely independent of the specific ester group substitution patterns, as these values tend to be the same for different ester-linked analogues that bear the same aryl ring substituents.

Review of the crystallographic environments of these structures revealed that whereas the minimum surface contacts defining the van der Waals envelope were remarkably similar for all 33 compounds studied, the directionalities of hydrogen bonds experienced by these molecules varied, even within groupings with common ester group orientations. It was noted that the ester groups of these molecules could form hydrogen bonds only to their keto oxygen atoms and not to their

ether-linked oxygens. Furthermore, the keto oxygens of ortho-phenyl substituted analogues could only form hydrogen bonds in the *sp* orientation, as the *ortho*-substituent shielded the bowsprit end of the molecule to prevent hydrogen bond formation to the ester in the *ap* conformation. In all 33 structures the DHP amine group was found to form a well-defined hydrogen bond in which the proton acceptors for the  $sp^2$  hybridized DHP amine were colinearly directed along the line of sight of the N(1)-H bond and clustered in a small volume of space. This contrasted markedly with the spatial patterns of hydrogen bond donors to the keto-ester oxygen atoms which were not so tightly clustered on the molecular surfaces generated from the crystallographic data.

The above observations indicated that the 1,4-DHP receptor probably recognizes nifedipine antagonists by means of a hydrophobic cleft that fits the phenyl ring and upper face of the aryl-DHP van der Waals envelope. This template would properly orient the molecule to form hydrogen bonds to peptide donor and acceptor groups located around the edge of this recognition surface. Given that the aryl ring would be immobilized, it was shown that the degree of DHP pucker would directly affect the angle at which the DHP amine group could form a hydrogen bond (Fig. 2) to its binding site in the receptor surface. The more active nifedipine analogues, which have flatter DHP rings, would have the DHP amine proton positioned to form a hydrogen bond with the amine acceptor group in the receptor cavity. Weakly active analogues would not have the amine proton pointing in this exact same direction, but approximately 2.0 Å higher in the receptor cavity, as a consequence of the greater degree of DHP ring puckering (Fig. 2). This requirement for the DHP amine to form hydrogen bonds with the receptor is consistent with SAR studies which indicate that the N(1)-H group is crucial for calcium channel activity, and the replacement of the

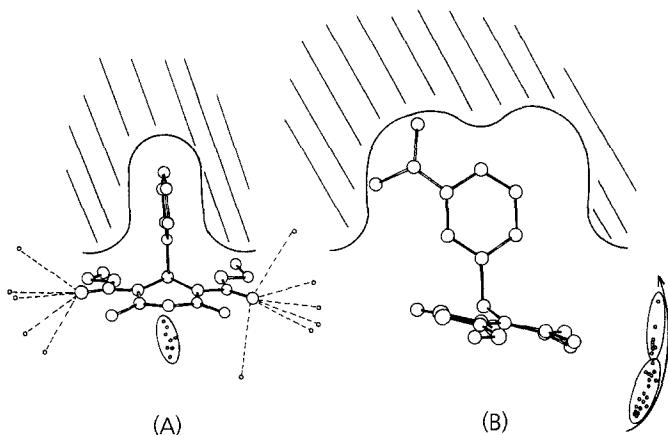


Fig. 2. Cartoon illustrating the mode of ligand binding to the DHP receptor surface. (A) Caudal view depicting the hydrophobic cleft fitting the aryl ring and upper surface of the DHP ring. Hydrogen bond donors to the keto-oxygen atoms of *sp* oriented esters can presumably lie anywhere in the vertical planes of the carbonyl groups, here identified by dashed lines to donor atom positions taken from eight antagonist crystal structures. The positions of hydrogen bond acceptors for the DHP amine proton are clustered and represented by the eight small circles seen within the oval at the amine end of the molecule. (B) Lateral view depicting the fit of the cleft to the aryl ring. The cleft is shaped to prevent *para*-substituted analogues from fitting. The positions of the DHP amine acceptors taken from the crystal structures of 19 strong and 8 weak antagonists are indicated in two ellipses in the right-hand-side of the figure. The different degrees of DHP ring puckering are seen to resolve the positions of these acceptors for the weak and strong antagonists into two separate clustered groups. It may be inferred that the receptor has a fixed hydrogen bond acceptor ligand in the vicinity of the lower ellipse.

amine proton by an alkyl group or its removal by oxidation of the 1,4-DHP to a pyridine ring abolishes activity [17].

#### *Characteristics of the voltage-sensing unit*

Based on the published amino acid sequences of the channel-forming  $\alpha$ -subunits of voltage-gated channels, several models have been proposed to describe how the putative transmembrane  $\alpha$ -helical segments may be arranged in the lipid bilayer [26-28]. These ion channel sequences are characterized by four homologous repeating domains which are organized around a common ion-selective channel. Each of the four domains of the protein sequence contains eight putative  $\alpha$ -helices, and the fourth helix in each domain, often referred to as the S4 helix, has a regular pattern of five or six positively charged Arg or Lys residues spaced every third amino acid apart.

Each residue in an  $\alpha$ -helix is displaced by a 100-degree right-hand rotation from its preceding N-terminal residue; thus a three-residue separation corresponds to a 300-degree right-hand, or 60-degree left-hand, helical displacement. A periodic pattern of five or six Arg and Lys residues, each spaced three apart, would form a left-handed spiral staircase of positive charges about the S4 helix. This spiral staircase of charged Arg and Lys side chains is proposed to hydrogen bond the S4 helix to a matching helical pattern of hydrogen bond accepting residues provided by the outer sur-

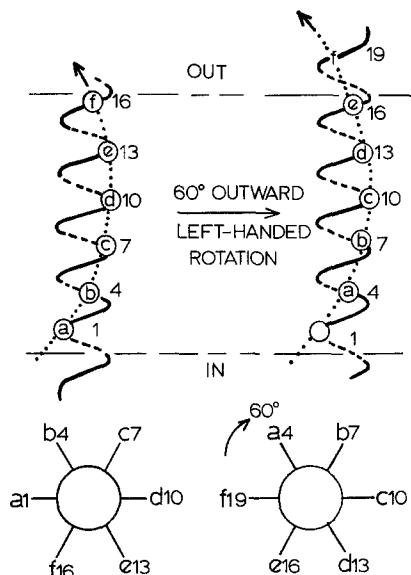


Fig. 3. Schematic illustration of the sliding helix model for voltage sensation across the membrane bilayer (adapted from Catterall [26]). The numbers 1 through 16 (mod 3) indicate the relative heights of fixed hydrogen bond acceptor groups (open circles) within the bilayer. Positive-charged Arg and Lys side chains on the S4 helix are assigned the letters a through f and positioned within the circle of the acceptor group with which they are hydrogen-bonded. The mechanism of voltage sensing involves a 60-degree left-handed outward displacement of the S4 helix in response to membrane depolarization. The dotted line with an arrow head indicates the direction of the helical displacement. The lower part of the illustration shows axial views of the helix as seen from the extracellular side of the cell membrane. The effective end result of the single step helical displacement is to move the positive charge at a1 on the cytoplasmic side of the membrane to f19 on the extracellular side.

rounding collar of  $\alpha$ -helices in each voltage-sensing bundle. It has further been proposed that the S4 helix is able to slide up or down in this semi-rigid collar of the sensing unit in response to changes in the membrane potential. The staircase of charged Arg and Lys residues can be placed in register with the next adjacent hydrogen bonding sites provided by the collar by simply twisting the S4 helix in the direction of this staircase as described in Fig. 3.

The slip-trough distance between two Arg side chains related by this helical rotation of the sensor is approximately 10.5 Å, and corresponds to a 5.0 Å axial shift coupled with a 9.0 Å left-handed displacement of the guanidyl terminus of the side chain relative to the S4 helical axis. If the Arg and Lys residues are directly hydrogen-bonded to groups on the outer sensing collar of helices, in what may be called the eclipsed hydrogen-bonded configuration of the sensor, transitions between sensor state configurations must involve a translocation of the S4 helix in a non-hydrogen-bonded state for a good portion of the 10.5 Å pathway. Changes in membrane potential will induce the S4 helix to move normal to the bilayer, but it is unclear as to what forces will be strong enough to cause the helix to undergo a left-handed twist over a 9.0 Å distance to prevent the sensor from being untracked from the helical sliding trough.

#### *Putative 1,4-DHP receptor sites on the S4 sensor*

$\text{Ca}^{2+}$  channel drugs are thought to interfere directly or indirectly with the voltage-induced translocations of the S4 helix. Recalling the ligand/receptor characteristics cited above, it may be possible to extend our analysis to postulate the nature of the groups which may be involved in defining those interactions with the drug molecule. The DHP amine function should interact with proton accepting functional side chains of such amino acids as Asp, Asn, Glu, Gln, Ser and Thr, while the ester keto-oxygens should seek to form hydrogen bonds with such proton donors as Arg, Lys and His. Given the scarcity of Lys, Arg and His residues on all the other putative transmembrane helices, and their proposed role as hydrogen-bonding donors to the receptor-bound drug, it appeared reasonable to investigate whether a nifedipine analogue could satisfy any of its receptor binding needs by interacting with the S4 sensor strand. CPK models suggested that the keto-ester oxygens of a drug molecule could hydrogen bond to adjacent Arg-X-X-Arg side chains which would be in proximity to one another as a result of a 300-degree turn of the  $\alpha$ -helix. Here the symbol X represents any generally hydrophobic amino acid residue such as Ala, Val, Leu, Ile or Phe. There clearly are multiple, potential Arg-X-X-Arg sites on any S4 strand where such recognition could occur, but it is also clear that drug binding at two adjacent sites might not be favorable since it may be difficult to position the central, shared Arg side chain to form simultaneously good hydrogen bonds to neighboring drug molecules.

These model building studies were further explored on a graphics system as shown in Fig. 4. The resultant drug/receptor model is seen to insert the aryl ring of the drug molecule edgewise into a cleft formed by the helical groove of the S4 strand. The unsubstituted *para* position of the phenyl ring forms tight van der Waals interactions with the core of the helix, thus explaining why *para*-phenyl substitutions abolish antagonist potency and receptor binding [17]. The aliphatic connecting arms of the two Arg side chains flank opposite sides of the phenyl ring as they reach down and back over the molecule to position their terminal N( $\eta$ )-H moieties in acceptable hydrogen bonding geometries with the two *sp,sp* oriented keto-ester oxygen atoms. Lys side chains appear to be too short to form hydrogen bonds in this manner. Moreover, the planes of the  $\pi$ -resonant guanidyl groups of the chelating Arg residues stack parallel, ~3.4 Å on either side of the aryl

ring of the DHP drug molecule. Such planar stacking arrangements have occasionally been observed for guanidyl side chains in crystal structures [29], but by far the most dominant interaction preferred by this moiety involves strong hydrogen bond formation. When the molecule is held in the helical groove, as shown in Fig. 4, the N(1)-H bond of the DHP amine function is approximately perpendicular to and directed outwards away from the axis of the S4 helix toward a hydrogen bond acceptor, presumably located on an adjacent helical strand in the voltage-sensing bundle.

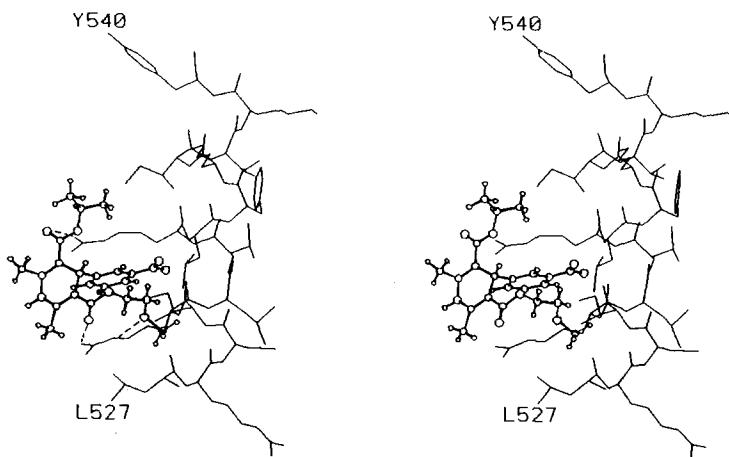


Fig. 4. SYBYL-generated stereodiagram of nimodipine bound to the receptor surface provided by the S4 strand from domain II of the  $\alpha_1$  subunit from rabbit skeletal muscle T-tubule membranes. The sequence shown extends from Leu<sup>527</sup> to Tyr<sup>540</sup> (residues RLCFRLRLFKITY), the aryl ring of nimodipine is docked in the cleft between Arg<sup>531</sup> and Arg<sup>534</sup>. The direction of the N(1)-H DHP bond is approximately perpendicular to and pointing outwards from the  $\alpha$ -helix axis which is oriented vertical in the plane of the page.

#### *Molecular basis of drug tissue selectivity*

The two ester groups of an antagonist molecule may be presumed to fit into specific lipophilic pockets in the DHP receptor when the binding orientation of the molecule is keyed on the joint conformation of the aryl and DHP rings. The tissue selectivity exhibited by certain analogues may be, in part, a consequence of differences in volume, shape and lipophilic character of these ester-sensing pockets among the various excitable tissues. An inspection of our drug/receptor model reveals that the ester groups of the drug molecule slide under the aliphatic connecting arms of the Arg side chains and rest against the side chain residues of amino acids exposed in the next turn of the helix. More specifically, given a typical peptide sequence such as Arg<sup>531</sup>-Leu-Leu-Arg-Leu-Phe-Lys-Ile, that is observed for the S4 strand in the second homologous domain of the  $\alpha_1$  subunit, if a nifedipine analogue were to bind with its corresponding port and starboard keto-oxygens to Arg<sup>531</sup> and Arg<sup>534</sup>, their ester groups would be seen to nestle against the side chains of Leu<sup>535</sup> and Ile<sup>538</sup>, respectively, as shown in Fig. 5.

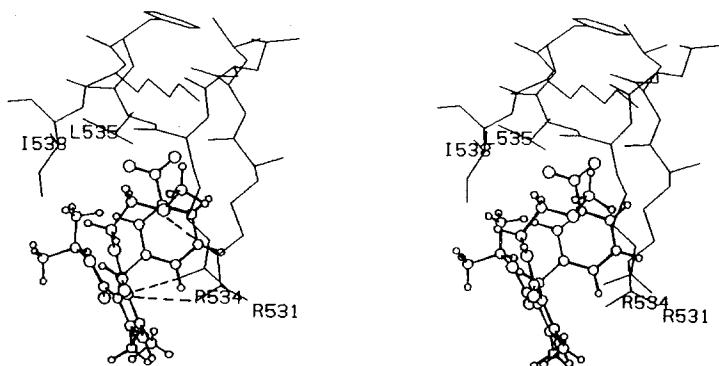


Fig. 5. Close up sideways view of nimodipine bound to the Arg<sup>531</sup>-Leu-Leu-Arg-Leu-Phe-Lys-Ile reading frame. Leu<sup>535</sup> and Ile<sup>538</sup> lie in the ester-sensing pockets of the receptor surface. Hydrogen bonds between the Arg<sup>531</sup> and Arg<sup>534</sup> side chains and the drug molecule are indicated (see also Fig. 6A). Note that Arg<sup>531</sup> forms bonds to both the keto-oxygen and the terminal ether oxygen of the nimodipine 2-methoxyethyl side chain, as further illustrated in Fig. 6C, and that the side chain has essentially the same conformation as shown in Fig. 1B. The direction of the helix axis is horizontal in the page.

Attempts were made to bind the molecule to the helix in an alternative orientation which involved fixing the aryl ring in the cleft and rotating the rest of the molecule 180 degrees around the C(4)-C(7) bond that connects the aryl ring to the 1,4-DHP ring. This new orientation would now bind the port keto-oxygen of the nifedipine analogue to Arg<sup>534</sup> and its starboard keto-oxygen to Arg<sup>531</sup>, but our modeling efforts revealed that the Arg-connecting arms may be too short to form good hydrogen bonds in this alternate binding geometry. Moreover, tissue-selective antagonists that have 2-alkoxyethyl ester groups, such as nimodipine and its bis(2-isopropoxyethyl) ester analogue, niludipine, appear to be able to form additional ester-linked hydrogen bonds from the 2-oxyethyl ether oxygen atoms to the secondary N( $\epsilon$ ) amine nitrogens of the Arg side chains (Fig. 5). Given the constraints of our initial drug/receptor binding model, we find that the upturned conformation observed for the 2-methoxyethyl side chain in the crystal structure of nimodipine (Fig. 1B) is precisely that required to form this additional bond. We further note that these additional bonds draw the terminal ester alkyl groups into closer proximity with residues Leu<sup>535</sup> and Ile<sup>538</sup>, allowing for a tighter conformational discrimination with regard to the nature of the groups involved. Space-filling molecular illustrations depicting the binding of the Arg side chains to the keto-oxygens of nimodipine, the fit of the 4-aryl ring substituent into the hydrophobic cleft, the association of the ester groups with Leu<sup>535</sup> and Ile<sup>538</sup>, and the bidentate binding of Arg<sup>531</sup> to the 2-methoxyethyl ester side chain are given as Figs. 6A, B and C.

One of the referees called our attention to the fact that nicardipine, which has a methylbenzyl-aminoethyl ester group, can be positioned similar to nimodipine in the binding cleft such that its tertiary side chain amine faces the secondary N( $\epsilon$ ) amine of Arg<sup>531</sup>. At physiologic pH the side chain amine will be unprotonated and capable of accepting a hydrogen bond from the N( $\epsilon$ )-H proton, but the benzyl and methyl amine substituents of the nicardipine side chain may be a bit too bulky to fit into the starboard Ile<sup>538</sup> binding pocket. It remains to be determined whether the S4  $\alpha$ -helical sequences of other tissue sources may have less bulky functional groups defining the

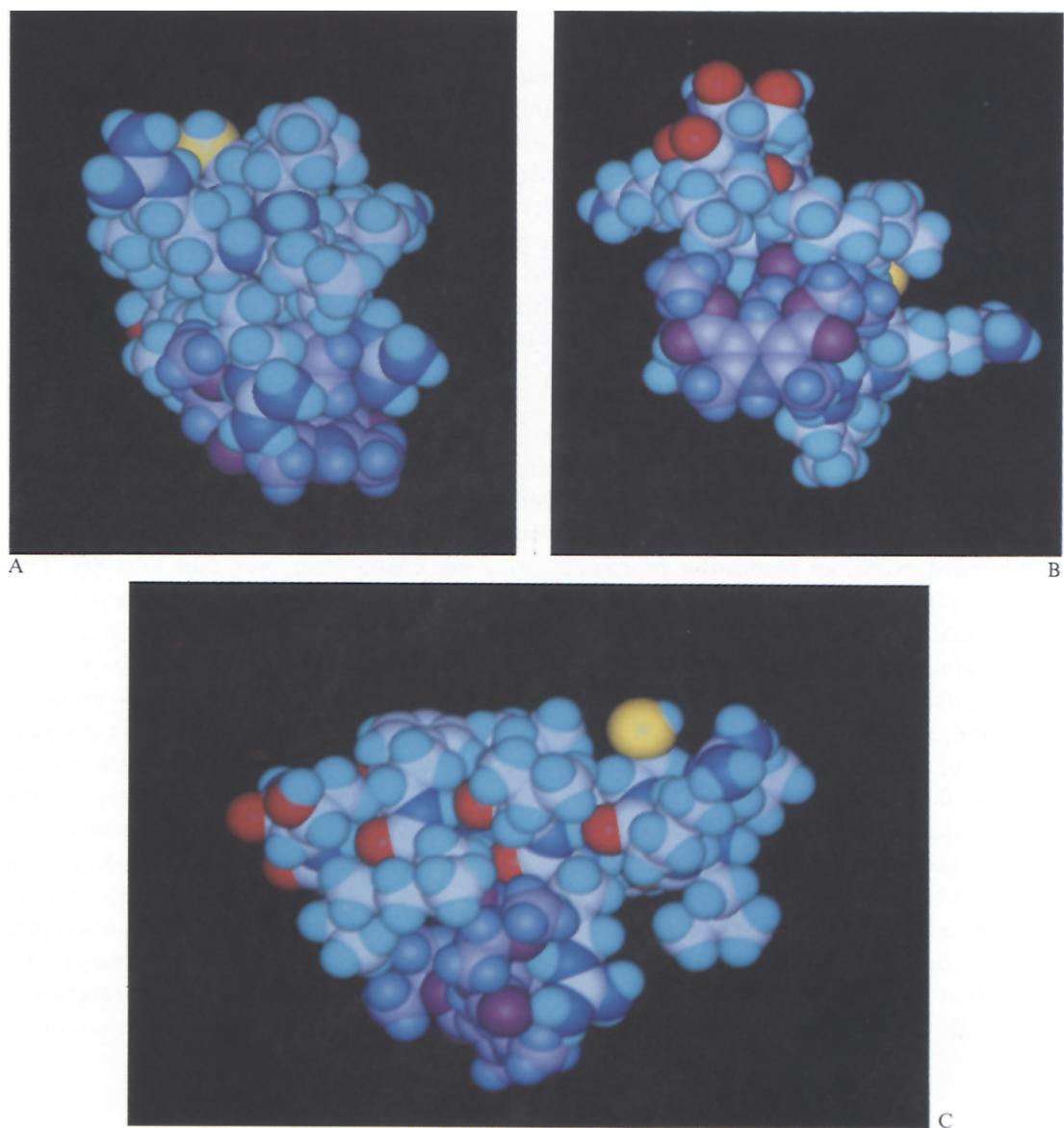


Fig. 6. Space-filling illustrations of the helix-drug interactions. (A) Dorsal view depicting the Arg binding to the keto-oxygens of the ester groups and the fit of the 4-aryl ring substituent into the hydrophobic helical cleft, (B) bottom and (C) side views showing the fit between the ester group alkyls and the Leu<sup>535</sup> and Ile<sup>538</sup> side chains which face the respective upper right- and left-hand-side ester groups in B and C. In panel C the bidentate binding of Arg<sup>531</sup> with the 2-methoxyethyl side chain of the nimodipine molecule is illustrated.

starboard ester pocket which would be more conducive for niludipine and nicardipine to form this auxiliary N( $\epsilon$ )-H bond.

Diethyl ester groups may represent the minimum bulk size and linear chain extension to generate significant lipophilic interactions with the amino acid side chains that protrude into the ester binding portion of the receptor cavity. There are a total of ten distinct Arg-X-X-Arg-P-X-X-S reading frame sites located on the four S4 strands of the  $\text{Ca}^{2+}$  channel subunit. Six of the ten sites are unique with regard to the particular identity of the amino acid residues in the port (P) and starboard (S) ester pockets; only two pairs of sites are found to have repeating patterns (Leu, Leu and Val, Pro). If it is assumed that  $\text{Ca}^{2+}$  channels for different tissues will exhibit a different spectrum of ester sensing residues, it will be a challenge to devise a strategy for selective drug action based on known S4 protein sequences and relevant affinity data for the different kinds of isolated binding sites.

#### *The eclipsed voltage sensor model*

At this juncture it may be instructive to examine what the eclipsed hydrogen-bonded configuration of the S4 helix has to offer with regard to binding 1,4-DHP drugs for the various gating states of the voltage-sensing unit. Prior to the ligand-binding event, the Arg side chains are directly hydrogen-bonded to various permissible proton-accepting side chains (Asp, Asn, Glu, Gln, Ser, Thr) extending from adjacent helices in the voltage-sensing bundle. Nifedipine drugs must first be able to dissociate the hydrogen bonds for an adjacent pair of Arg sites if it is to bind at that location on the S4 sensor. A consideration of secondary but equal importance is whether a DHP amine acceptor group is available to bind to the drug to complete the proposed receptor site geometry when the ligand binds to the Arg-X-X-Arg cleft in this manner. A third consideration concerns whether or not this receptor binding event stabilizes or destabilizes the initial gating state configuration of the voltage-sensing unit. The popular consensus is that nifedipine drugs prolong the lifetimes of the gating states to which they prefer to bind. With regard to these three considerations, eclipsed gating state configurations of the S4 sensor are not stabilized by ligand binding. Two hydrogen bonds between the S4 rotor and its collar must be broken, ligand binding does not restore these connections, and none of the allowed eclipsed rotor conformations places an acceptor group facing the DHP drug to bind to its amine group. And also, as noted above, it is not clear as to what forces will ensure that the S4 helix will undergo the required left-handed rotation to prevent the S4 rotor from being untracked as it jumps from one gating state configuration to the next.

#### *The staggered voltage sensor model*

In contrast to these observations, a staggered S4 rotor configuration appears to satisfy many of the shortcomings of the eclipsed rotor model. The staggered rotor model presumes that the steps between rotor state positions do not involve abrupt electrostatic discontinuities in the manner in which charged groups on the radial periphery of the S4 helix twist and jump the 10.5 Å distance from one rotor state to the next. The staggered rotor configuration places the proton-accepting groups from the collar of the sensor symmetrically midway between the adjacent pairs of Arg and Lys residues on the S4 helix, or about 5.5 Å apart (Fig. 7). Intervening water molecules could easily bridge this 5.5 Å distance and form reasonable hydrogen-bonded contacts ( $\sim 2.75$  Å) between these donor and acceptor groups which line the helical sliding trough of the S4 rotor. The bident-

ate width of the carboxylate ( $-O-C-O-$ ) and guanidyl ( $-H_2N-C-NH_2-$ ) functional groups will ensure that the water molecules can bridge the gap should these dimensions dilate and compress as the rotor jumps from one energy minimum state to the next. Most importantly, the maintenance of these hydrogen-bonded contacts would ensure that the S4 rotor would not become derailed as it attempted to jump from one sensing state position to the next. Drugs binding to the staggered sensor configuration (i) do not weaken any bonds between the S4 helix and its collar, (ii) the collar of the sensing unit has acceptor groups ideally positioned for bonding to the DHP amine of nifedipine analogues when they insert into the Arg-X-X-Arg binding cleft, and (iii) the number of stabilizing bonds between the rotor and its collar are increased as a consequence of the ligand binding event. Although the amine acceptor groups, which are extended amino acid side chains, have a certain degree of lateral flexibility in forming hydrogen bonds to the DHP amine, the 2.0 Å displacement indicated in Fig. 2B for differentiating between strong and weak antagonists may be sufficiently large to prevent these acceptors from shifting far enough to strongly bind the more highly puckered weak antagonists.

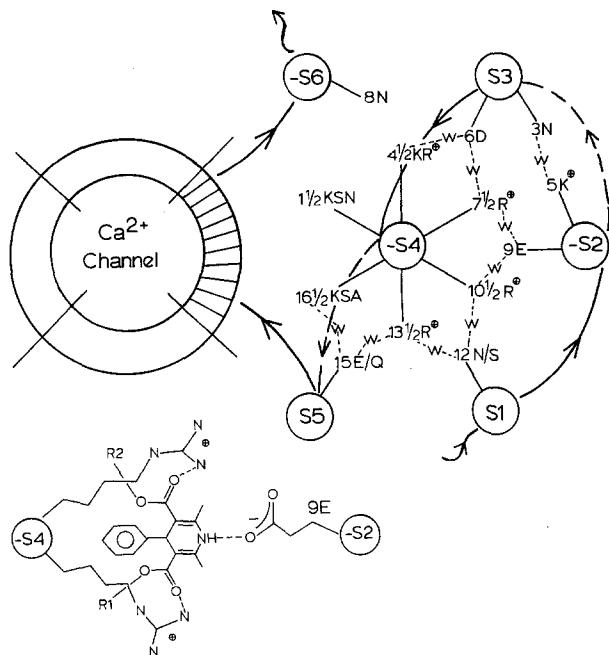


Fig. 7. Extracellular view depicting the arrangement of  $\alpha$ -helices for the staggered rotor model of the voltage-sensing unit (see axial view of Fig. 3 for comparison). Helix labels S1 through S6 correspond to the assignments of Tanabe et al. [7], negative signs associated with helices S2, S4 and S6 indicate their directions have been reversed to allow them to pass back to the cytoplasmic side of the membrane. Transmembrane heights and radial positions of the hydrogen-bond-forming amino acid side chains are indicated by the one letter symbol of the residue and a number (1-20) representing the relative distance from the cytoplasmic side of the bilayer. Water molecules ( $-W-$ ) bridge the gap between the Arg and Lys groups on the S4 strand and hydrogen bond accepting groups on the surrounding helices. The helical sense of the voltage sensor is that of an outward, left-handed, 60-degree rotation of the S4 helix in response to membrane depolarization as described in Fig. 3. The inset detailing helices S2 and S4 indicates how nifedipine analogues may bind to stabilize a particular gating state of the voltage-sensing unit.

One criticism of the staggered rotor model, however, is the large number of potential binding sites on the four S4 sensors associated with each of the four domains of the protein. Ester group interactions may eliminate certain binding sites from consideration, but it is not obvious as to how a particular binding site among those remaining would have the enhanced binding affinity required to be consistent with the competitive inhibition observed in radioligand binding experiments. That such high affinity sites exist for the  $\text{Ca}^{2+}$  channel protein may be inferred from the fact that nifedipine drugs, both antagonists and activators, bind to and similarly affect the structurally homologous  $\text{Na}^+$  channel [30], albeit at concentrations that are three orders of magnitude larger than that required for  $\text{Ca}^{2+}$  channel binding.

#### *Other tertiary structural considerations*

Although hexagonal packing patterns have been observed for helical structures in nature, modeling studies of voltage-gated ion channels [27,28] have generally incorporated the four- $\alpha$ -helix bundle [31] as a structural motif. The four- $\alpha$ -helix bundle is a pattern of association often found in globular protein structures, but it remains to be determined whether this pattern of association will be a common feature of helix bundles that traverse membrane bilayers. The bacterial photosynthetic reaction centers from *Rhodopseudomonas viridis* [32] and *Rhodobacter sphaeroides* [33], which represent the only integral membrane protein structures which have been determined at atomic resolution, have an homologous transmembrane structure consisting of a cluster of eleven transmembrane  $\alpha$ -helices, the core of which is a bundle of four  $\alpha$ -helices. The remaining seven helices that are arranged around this core are irregularly placed around the bacteriochlorophyll, quinone and carotenoid cofactors, and as such do not form a close-packed network (see Ref. 32, Fig. 8). Given that the primary amino acid sequences indicate that the channel-forming  $\alpha$ -subunits of the calcium and sodium channels are each organized into four homologous voltage-sensing domains [34,35], modeling studies have generally organized these four voltage-sensing units around a central ion channel which has approximate four-fold symmetry. Four hexagonally packed sensor clusters, as shown in Fig. 7, could be perturbed when positioned around an ion channel possessing four-fold symmetry. But whether this implies that the voltage sensor itself will transform into a tetragonal arrangement of helices based on the four-helix bundle motif, or simply experience a deformation of the hexagonal packing pattern, are questions which remain to be answered. A deformation in the hexagonal arrangement of helices around the S4 voltage sensor may offer an explanation as to how certain reading frames could become stronger ligand binding sites than others. The hydrogen bonding of the S4 rotor to its collar would neither be exclusively eclipsed nor staggered, but would require a combination of both direct and staggered, as well as intermediate type bonds, with the end result that very few reading frames would have two staggered hydrogen bond attachments to the collar and predispose it to bind nifedipine ligands with high affinity.

## CONCLUSIONS

The present diffraction study indicates that large differences in the bulk sizes of the two ester substituents may not select a specific *sp,ap* ester conformation that would place the bulkier ester group in the less sterically hindered *trans* orientation, and predispose the molecule for receptor binding. The *cis*↔*trans* isomerization of the ester groups does not appear to markedly perturb the degree of puckering of the DHP ring, which suggests that tissue selectivities are largely determined

by the nature of the two ester groups. A receptor binding model has been described which may, in part, explain the basis of tissue selectivity for the 1,4-DHP  $\text{Ca}^{2+}$  channel antagonists and how these drugs could interfere with voltage-sensing processes at the molecular level. The model differs markedly from an earlier molecular detailed study [25] in four important respects: (i) the 1,4-DHP aryl ring lies in an aliphatic cleft of an  $\alpha$ -helix, and is shielded from interactions with other amino acid side chain residues, (ii) both ester groups are in a *cis* or *sp* conformation, (iii) the ester groups of the drug interact primarily with aliphatic amino acid side chains rather than aromatic ones, (iv) the drug is held locked into the surface of the receptor by three or more well-defined hydrogen bonds. The receptor binding model is not compatible with an eclipsed voltage-sensor configuration, but rather with a staggered rotor conformation that has certain desirable characteristics in which the eclipsed model may be deficient. A reduction in the hexagonal symmetry of the voltage sensors would require the S4 strand to be linked to its collar by a combination of both eclipsed and staggered hydrogen bond attachments which would reduce the number of reading frames that are accessible to ligand binding.

The binding model which has been presented is sufficiently detailed to provide useful three-dimensional coordinates to describe the major interactions between the ligand and the S4 sensor. The relative position of the amine acceptor group may be inferred from the docked ligand, but the architectural details as to the conformation of the amine acceptor side chain on its helix and the exact positioning of the other  $\alpha$ -helices which describe the voltage-sensing bundle cannot be as reliably reported at this time. Molecular mechanics procedures may be useful to verify the reported activities of different nifedipine analogues by estimating their relative binding affinities to various potential binding clefts on the S4 strand. These calculations for the present, however, must either neglect or imprecisely model the important contribution that the amine acceptor adds to the stability of the complex. Moreover, it is not known which of the S4 binding clefts from the rabbit skeletal sequence may be associated with ligand binding, nor have the differences in the amino acid sequences of the S4 strands of most tissues of pharmacologic interest been determined. The reader should be cautioned that although this study has produced a binding-site model which has intriguing pharmacologic possibilities, the degree to which this model may be correct has yet to be established. Biochemical methods such as affinity labeling and site-directed mutagenesis may be expected to resolve certain of the questions raised in this structure-activity/molecular modeling investigation, and this binding-site model may play an important role in the design of experiments to further probe this issue.

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#### REFERENCES

- 1 Triggle, D. J., Langs, D. A. and Janis, R. A., Med. Res. Rev., 9 (1989) 123.
- 2 Kazda, S., Garthoff, B. and Knorr, A., Fed. Proc., 42 (1983) 196.
- 3 Towart, R., Wehinger, E., Meyer, H. and Kazda, S., Arzneim.-Forsch./Drug Res., 32 (1982) 338.

- 4 Deyo, R. A., Straube, K. T. and Disterhoft, J. F., *Science*, 243 (1989) 809.
- 5 Blessing, R. H., *Crystallogr. Rev.*, 1 (1987) 3.
- 6 Main, P., Lessinger, L., Woolfson, M. M., Germain, G. and Declerq, J. P., *MULTAN 77 – A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*, Universities of York, U.K. and Louvain, Belgium, 1977.
- 7 Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. and Numa, S., *Nature (London)*, 328 (1987) 313.
- 8 Fossheim, R., Swarteng, K., Mostad, A., Romming, C., Shefter, E. and Triggle, D. J., *J. Med. Chem.*, 25 (1982) 126.
- 9 Triggle, A. M., Shefter, E. and Triggle, D. J., *J. Med. Chem.*, 23 (1980) 1442.
- 10 Hempel, A. and Gupta, M. P., *Acta Crystallogr.*, B24 (1978) 3815.
- 11 Krajewski, J., Urbanczyk-Lipkowska, Z. and Gluzinski, P., *Acta Crystallogr.*, B33 (1977) 2967.
- 12 Krajewski, J. A., Urbanczyk-Lipkowska, Z. and Gluzinski, P., *Cryst. Struct. Commun.*, 6 (1977) 787.
- 13 Fossheim, R. J., *Acta Chem. Scand., Ser. B*, B39 (1985) 785.
- 14 Fossheim, R. J., *J. Med. Chem.*, 29 (1986) 305.
- 15 Fossheim, R. J., *Acta Chem. Scand., Ser. B*, B40 (1986) 777.
- 16 Fossheim, R., Joslyn, A., Solo, A. J., Luchowski, E., Rutledge, A. and Triggle, D. J., *J. Med. Chem.*, 31 (1988) 300.
- 17 Loev, B., Goodman, M. M., Snader, K. M., Tedeschi, R. and Macko, E., *J. Med. Chem.*, 17 (1974) 956.
- 18 Rodenkirchen, R., Bayer, R., Steiner, R., Bossert, E., Meyer, H. and Moller, E., *Naunyn-Schmied. Arch. Pharmacol.*, 310 (1979) 69.
- 19 Rodenkirchen, R., Bayer, R. and Mannhold, R., *Prog. Pharmacol.*, 5 (1982) 9.
- 20 Seidel, W., Meyer, H., Born, L., Kazda, S. and Domper, W., Abs. 14. Div. Med. Chem. American Chemical Society, 187th National Meeting, St. Louis, MO, 8-14 April 1984.
- 21 Baldwin, J. J., Claremon, D. A., Lumma, P. K., McClure, D. E., Rosenthal, S. A., Winquist, R. J., Faison, E. P., Kaczorowski, G. J., Trumble, M. J. and Smith, G. M., *J. Med. Chem.*, 30 (1987) 690.
- 22 Mannhold, R., Rodenkirchen, R. and Bayer, R., *Prog. Pharmacol.*, 5 (1982) 25.
- 23 Coburn, R. A., Wierzba, M., Suto, M. J., Solo, A. J., Triggle, A. M. and Triggle, D. J., *J. Med. Chem.*, 31 (1988) 2103.
- 24 Berntsson, P. and Wold, S., *Quant. Struct.-Act. Relat. Pharmacol., Chem. Biol.*, 5 (1986) 45.
- 25 Höltje, H.-D. and Marrer, S., *Quant. Struct.-Act. Relat. Pharmacol., Chem. Biol.*, 7 (1988) 174.
- 26 Catterall, W. A., *Science*, 242 (1988) 50.
- 27 Guy, H. R. and Seetharamulu, P., *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 508.
- 28 Greenblatt, R. E., Blatt, Y. and Montal, M., *FEBS Lett.*, 193 (1985) 125.
- 29 Kojic-Prodic, B., Razic-Toros, Z., Bresciani-Pahor, N. and Randaccio, L., *Acta Crystallogr.*, B36 (1980) 1223.
- 30 Yatani, A., Kunze, D. L. and Brown, A. M., *Am. J. Physiol.*, 254 (1988) H140.
- 31 Chou, K. C., Maggiora, G. M., Némethy, G. and Scheraga, H. A., *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 4295.
- 32 Deisenhofer, J. and Michel, H., *Science*, 245 (1989) 1463.
- 33 Feher, G., Allen, J. P., Okamura, M. Y. and Rees, D. C., *Nature (London)*, 339 (1989) 111.
- 34 Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. and Numa, S., *Nature (London)*, 312 (1984) 121.
- 35 Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. and Numa, S., *Nature (London)*, 320 (1986) 188.