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A molecular graphics study on structure-action relationships of calcium-antagonistic and agonistic 1,4-dihydropyridines

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SUMMARY

Based on force field and quantum chemical calculations a hypothesis on the molecular mechanism of Ca channel-modulating 1,4-dihydropyridines (DHPs) has been developed. A careful investigation of the molecular electrostatic fields of the compounds led to the discovery of a unique area of the molecular potentials where Ca agonists and antagonists possess potentials with opposite sign. It is further demonstrated that the molecular potential of a simple receptor site model is reduced by interaction with Ca channel-activating DHPs and on the contrary increased by Ca channel-blocking DHPs. It is concluded that these effects could be the basis for opposite actions of 1,4-dihydropyridine enantiomers at the potential-dependent Ca channels.

INTRODUCTION

Calcium ions play an important role in many biological processes. They function, for example, as electro-mechanic links in muscle cells like myocardium cells. When these are activated, a significant increase of intracellular calcium concentration occurs. A part of these calcium ions originates from intracellular stores, the rest streams from the extracellular space through potential-dependent calcium channels into the cells. Calcium antagonists inhibit these impulse-dependent transmembrane calcium fluxes. Molecules with the dihydropyridine (DHP) structure belong to the most active representatives of this group of drugs. The calcium influx-blocking property of DHPs has been known for about 20 years. Very recently, DHP molecules have been synthesized possessing calcium-agonistic action [1]. Further investigations with pure enantiomers showed that the *R*- and *S*-configurated enantiomers of one substance produce an opposite effect [2–4] at the

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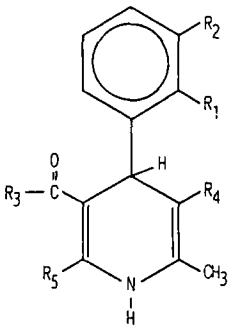
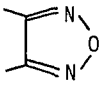
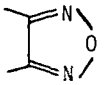
potential-dependent calcium channel. In this work, a hypothetical model is presented in order to give a possible explanation for the contradictory behavior of the enantiomers. The model was derived using quantum chemical and force field methods. A simple algorithm served for the characterization of electrostatic potential properties of calcium agonists and antagonists.

METHODS

As a first step, the molecular structures of six DHP derivatives (Table 1) were constructed in an Evans & Sutherland PS 350 system, applying the molecular modeling package SYBYL [5]. This list contains three chiral dihydropyridine derivatives with opposite action of the enantiomers, two chiral DHPs with antagonistic action of both enantiomers as well as one achiral DHP. Using the SEARCH-option of SYBYL, a conformational search for sterically-allowed, low-energy confor-

TABLE 1
STRUCTURE AND PHARMACOLOGICAL BEHAVIOR OF THE MOLECULES INVESTIGATED IN THIS STUDY



	R_1	R_2	R_3	R_4	R_5	Ca-Agonist	Ca-Antagonist
BAY K 8644	$-CF_3$	-H	$-OCH_3$	$-NO_2$	$-CH_3$	S	R
Sandoz 202 791			$i\text{-}OC_3H_7$	$-NO_2$	$-CH_3$	S	R
H 160/51	-Cl	-H	$-OC_2H_5$	-H	$-NH_2$	R(*)	S(*)
PN 200 110			$i\text{-}OC_3H_7$	$-COOCH_3$	$-CH_3$		R/S
Methyl-BAY E 6927	-H	$-NO_2$	$i\text{-}OC_3H_7$	$-COOC_2H_5$	$-CH_3$		R/S
Fossheim	$-CF_3$	-H	$-OCH_3$	$-COOCH_3$	$-CH_3$	(not chiral, Ca-Antagonist)	

* Predicted configuration

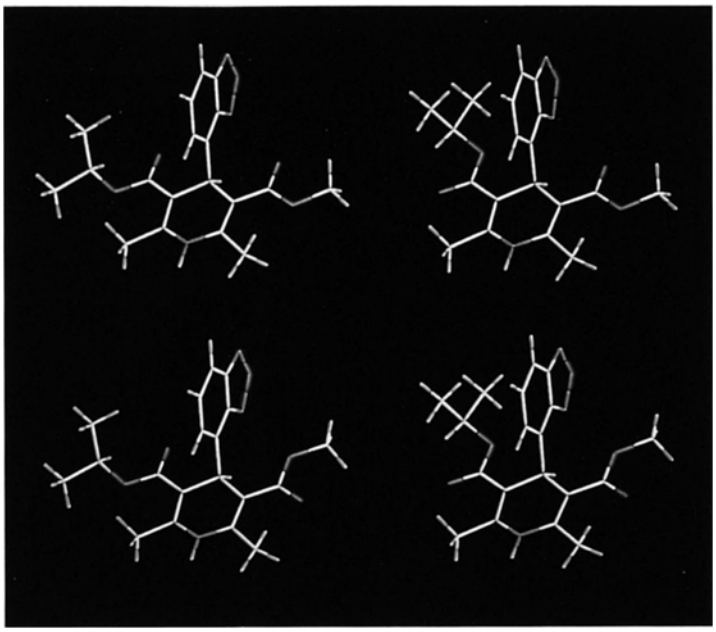


Fig. 1. The energetically preferred conformations of (*S*)-(+)-PN 200 110.

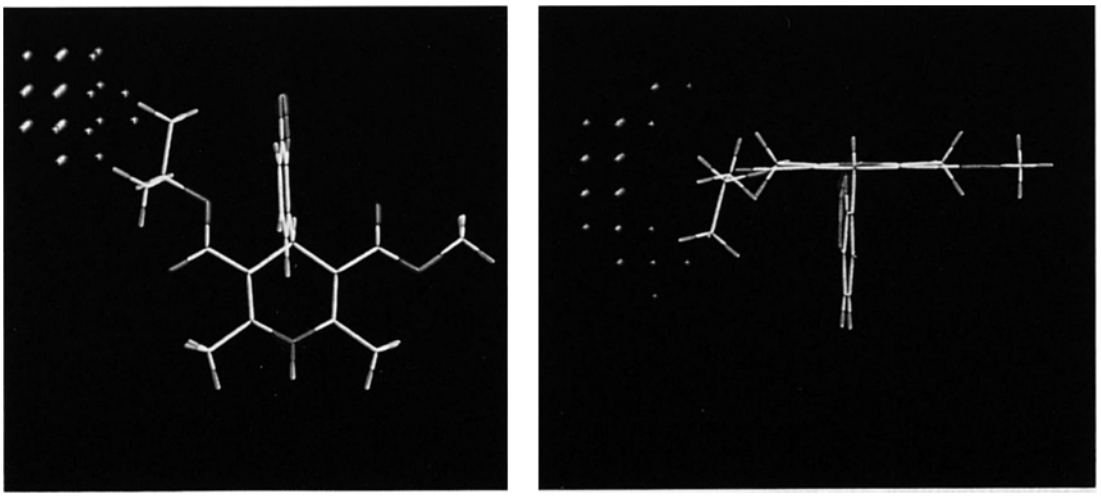


Fig. 2. In Fig. 2a, the type-coded structure represents (*S*)-(+)-PN 200 110. Magenta-colored crosses indicate the space where electrostatic potential differences between agonists and antagonists exist. To elucidate the spatial position of this area, Fig. 2b shows the 90° perspective relative to the X-axis.

mations was performed, neglecting the charge distribution. This analysis was divided into two parts. Firstly, the five main rotatable bonds were investigated using 30° increments. Then a second search using 5° increments was performed in the ± 30°-environment of low-energy conformers. Molecular energies were calculated and all conformations showing energy differences greater than 21 kJ/mol over the absolute minimum were discarded. Thereby, two preferred orientations were found for the ester substituents as well as for non *o*-trifluoromethyl-substituted phenyl groups. MNDO charge distributions [6] were then calculated for the most stable conformation of each conformational family. Finally, a geometry optimization was carried out using MAXIMIN, an optimization method within SYBYL which uses the Simplex-algorithm.

In the next step, electrostatic potentials were calculated for the optimized structures using the POTENTIAL-option of SYBYL. A positive test charge placed near a molecule equipped with MNDO point charges will be attracted or rejected, depending on the character of the molecular potential in the pertinent space segment. The resulting electrostatic potential can be calculated for each point in the space. In this work the calculation was made for a grid with an interval of 1 Å. Finally the calculated potentials of agonists and antagonists were analysed using SIMPOT [7]. SIMPOT is a program which can identify potential differences between two groups of molecules with a simple algorithm. The algorithm of this program searches for grid points with diverse electrostatic potential between the groups of molecules. The SIMPOT procedure possesses certain similarities to the DYLOMNS approach which was developed some years ago by Wise and coworkers [8].

Example	Grid point X	Grid point Z
Potential Agonist A	4.2 kJ/mol	4.8 kJ/mol
Potential Agonist B	4.7 kJ/mol	5.4 kJ/mol
Potential Antagonist A	6.3 kJ/mol	5.1 kJ/mol
Potential Antagonist B	6.9 kJ/mol	5.9 kJ/mol

In this example, the potentials of the agonistic and the antagonistic group of the DHPs differ in coordinate X, but not in coordinate Z. This means that the potential range of agonists and antagonists overlap at grid point Z, but not at grid point X. (The energy difference between both groups of molecules at X amounts to 2.1 kJ. From now on in this paper, such differences will be called 'potential differences'.) SIMPOT calculates statistical data for all grid points with different potentials as, for example, the lowest and the highest potential difference of each group of molecules, the potential difference between the two groups, and the average of the differences. Moreover, the potential energies of all grid points can be summed up specifically for each single molecule.

The molecular modeling and the force field calculations were performed on an Evans & Sutherland PS 350 graphics system as well as a Digital VAX 11/730 of the Pharmaceutical Institute, University of Bern. Quantum chemical calculations were done on an IBM 3038 of the Bernische Datenverarbeitungs AG, Bern.

RESULTS AND DISCUSSION

Conformational analysis and structure optimization do not result in the discovery of striking differences between calcium agonists and antagonists. Sp-rotamers (conformations with synperiplanar arrangements of the substituent at the aromatic system and the hydrogen atom in position 4) are energetically preferred for all investigated molecules. The ap-rotamers are at least 16.0 kJ/mol less stable than sp-rotamers. For a given spatial position of the phenyl substituent, the ester function can be orientated antiperiplanar or synperiplanar with respect to the double bonds of the DHP-ring (Fig. 1). In this case, energy differences between sp- and ap-conformations amount to maximally 11.7 kJ/mol. For five of the six compounds studied the ap-rotamer is energetically favored.

In order to form an idea of the possible mode of interaction of agonists and antagonists with the receptor, the preferred conformer of all molecules is positioned identically in cartesian space. This procedure is consistent with the fact that calcium channel activators and blockers are bound to the same receptor sites. All molecules are oriented in a way such that the DHP-ring is lying in the XY-plane and the aromatic system is pointing in the direction of positive Z-axis, so that it is located above the heteroring. If all structures are fixed in the described manner then it can be observed that agonists bear the space-consuming ester groups on the right side of the DHP-ring, whereas on the left side only non-ester substituents are found. On the contrary antagonists, whose corresponding enantiomers show opposite activity at the potential-dependent calcium channel, possess space-filling ester substituents on the left side. Finally, ester functions can be found on both sides if both the *R*- and *S*-configurations of the molecules act as antagonists or if the channel blockers are not chiral. These statements show that, in our approach, determination of the agonistic and antagonistic effect of the DHPs must be based on an interaction of the 'left' side of the molecules with the receptor at the calcium channel.

In fact, the SIMPOT program finds dramatic potential differences for the conformations listed

TABLE 2
CALCULATED ELECTROSTATIC POTENTIALS (in kJ) FOR THE ISOLATED MOLECULES (COLUMN 1), THE CORRESPONDING TRYPTOPHAN COMPLEXES (COLUMN 2) AS WELL AS EFFECTIVE POTENTIAL CHARGES (COLUMN 3)

Agonists	R_3	R_4	1	2	3
(*S*)-(−)-BAY K 8644	ap	–	−397.3	20.1	−316.1
(*R*)-H 160/51	ap	–	− 46.8	301.4	− 34.8
(*S*)-(+)-Sandoz 202 791	ap	–	−396.0	15.9	−320.3
Antagonists	R_3	R_4	1	2	3
(*R*)-(+)-BAY K 8644	sp	–	100.7	426.2	90.0
Fossheim	sp	ap	18.4	358.8	22.6
(*S*)-H 160/51	sp	–	56.8	381.0	44.8
(*S*)-(+)-PN 200 110	sp	ap	84.2	407.0	70.8
(*R*)-(−)-Sandoz 202 791	sp	–	178.9	483.6	72.4
(*S*)-(−)-Methyl-BAY E 6927	sp	ap	47.7	375.1	38.9

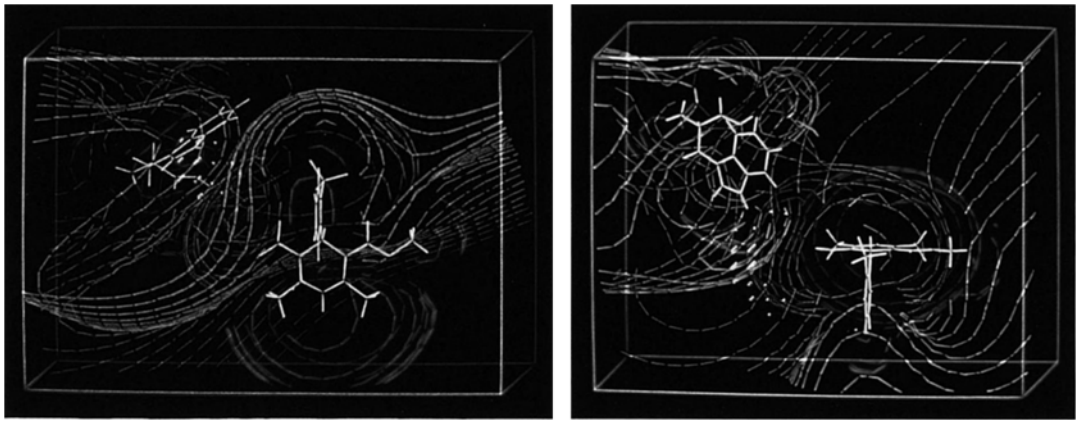


Fig. 3. Fig. 3 shows the relative position of tryptophan towards (*S*)-(−)-BAY K 8644 as an example of the investigated complexes.

in Table 2 at 35 directly adjacent grid points in this area. As the electrostatic potential was calculated with a raster of 1 Å, each grid point corresponds to a volume of 1 $Å^3$. Therefore the potential differences reside in a unique coherent volume of 35 $Å^3$ (Fig. 2). The average potential difference is −1.48 kJ/grid point, with a maximum of −5.69 kJ and a minimum of −0.01 kJ, respectively.

Significant potential differences can be observed between calcium channel activators and blockers, when the energy values of the 35 grid points are added for each molecule separately. As listed in Table 2 column 1, agonistic DHPs possess a strong negative molecular electrostatic potential in the specified area whereas the antagonistic DHPs have a positive potential in the same volume segment. It can be assumed that the opposite electrostatic fields of agonists and antagonists will influence the potential of the receptor protein in a different manner. Therefore, in a

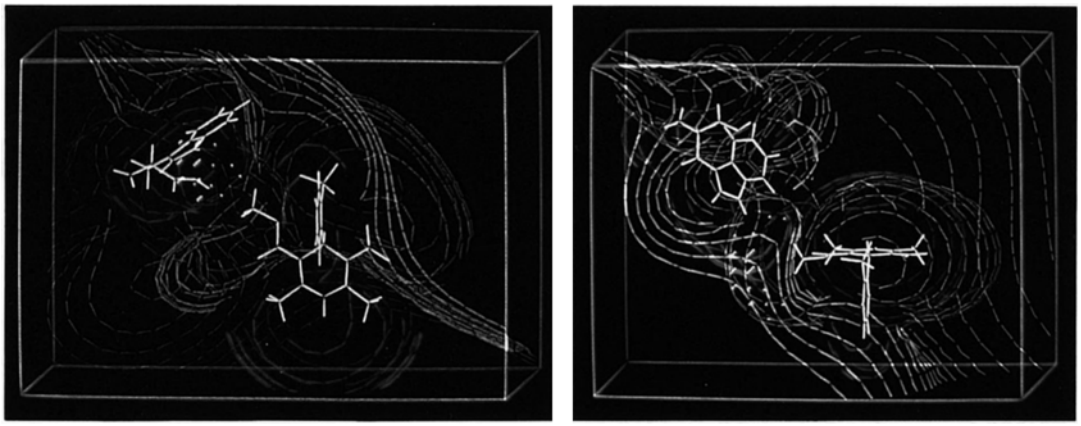


Fig. 4. Fig. 4a shows the electrostatic potential of tryptophan, contoured in the XY-plane. Red lines indicate a repulsive energy of +12 kJ/mol, blue ones an attractive energy of −12 kJ/mol, green represent neutral regions. Fig. 4 b shows the same molecule, but the isopotential lines are contoured in the XY-plane.

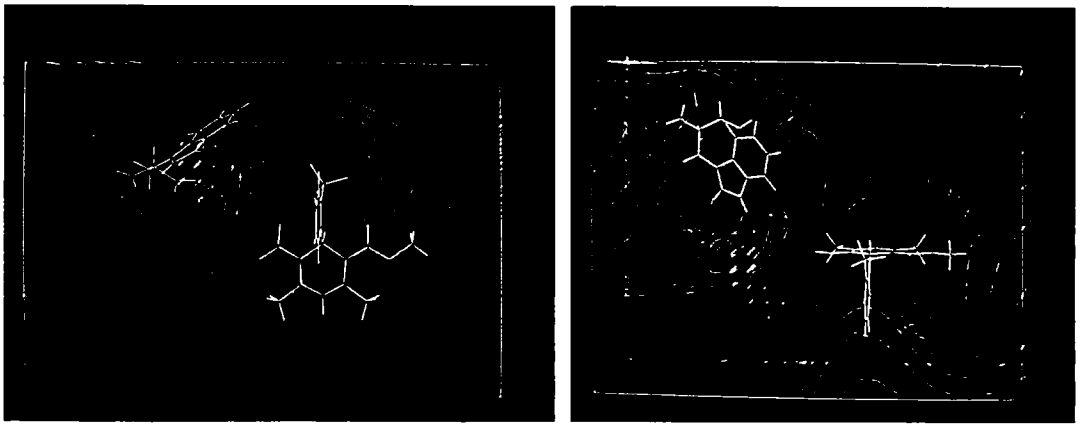


Fig. 5. Electrostatic isopotential lines for the tryptophan-(*S*)-(−)-BAY K 8644 complex. Contour levels: red, +12 kJ/mol, blue −12 kJ/mol, green 0 kJ/mol. Orange-colored crosses indicate the space where the potential of agonists and antagonists differ.

further step of our study, we examined how the electrostatic potential of the receptor is affected by both groups. Since, until now, no data concerning the structure of the receptor existed, tryptophan was used as a very simplified receptor model and the influence of calcium channel activators and blockers on this amino acid was examined.

The geometry of the interaction complex between tryptophan and the DHPs was chosen so that the tryptophan induces a positive electrostatic potential in that segment of space where the potential differences of agonists and antagonists reside (Figs. 3 and 4). Interaction energies for all complexes were calculated using the energy option of SYBYL and proved that the complex geometry

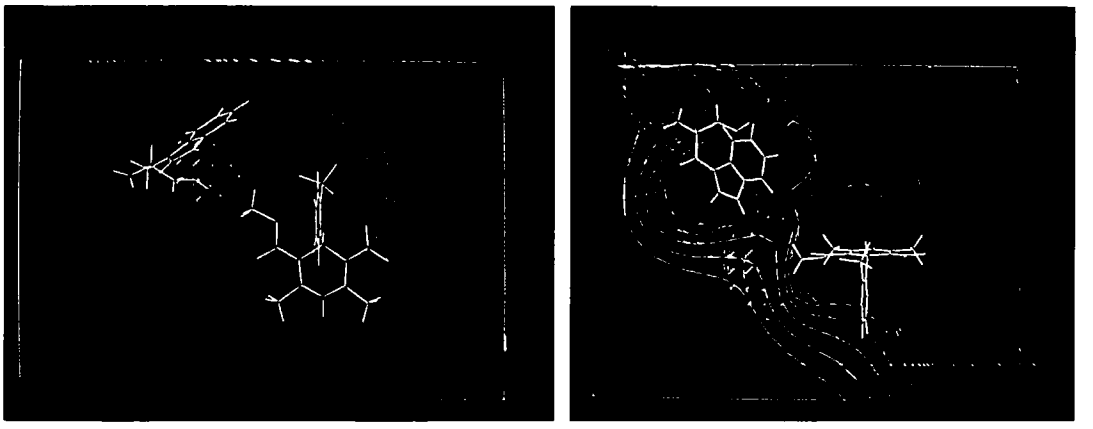


Fig. 6. As an example of a tryptophan-calcium antagonist complex, (*R*)-(+)-BAY K 8644 is displayed in Fig. 6. Energy levels of the isopotential lines are the same as in Fig. 5.

leads to attractive binding. The distance from the center of the DHP-ring to the center of the aromatic system of tryptophan is about 10 Å. SIMPOT was used to analyze the interaction potential of dihydropyridine-tryptophan complexes and it was found that potential differences between agonists and antagonists exist at 28 grid points. The mean value of the interaction potential difference is -1.65 kJ/grid point ($s = 1.66$), the highest difference is -5.69 kJ, the lowest one -0.03 kJ. These values are the same order of magnitude as the corresponding data from the isolated DHPs. Therefore it can be concluded that the molecular electrostatic potential of tryptophan is influenced in direct proportion by the Ca channel-modulating dihydropyridines.

The isolated tryptophan model induces a potential sum of 336.2 kJ at the 28 grid points mentioned above. After complex formation with agonists (Fig. 5), negative potential sums are found in this area, while corresponding values for antagonists (Fig. 6) are positive (Table 2 column 2). Subtraction of the potential sum of tryptophan alone and the complexes gives the effective potential changes. These values show a clear-cut distinction between agonists and antagonists; agonists reduce the receptor potential and antagonists increase it (Table 2 column 3).

CONCLUSION

In this study, significant differences were found in a specific spatial segment of the molecular electrostatic fields of calcium agonists and antagonists. It was further shown that calcium channel activators and blockers can exert an opposite effect on the electrostatic potential of a receptor protein. The potential of tryptophan, simulating a simple receptor model, was decreased through agonists and increased through antagonists. We, therefore, come to the conclusion that the molecular electrostatic potential of the DHP-binding site can be influenced in opposite ways by binding of calcium channel modulating molecules with DHP structure. Ca channel activators decrease the electrostatic potential. In our view, this change affects the structure of the calcium channel proteins resulting in an increased influx of calcium ions into cells. Ca channel blockers, on the contrary, increase the electrostatic potential at the receptor, thereby triggering a structural change of the channel which leads to a reduction of calcium influx. The validity of this hypothesis will be challenged by each new chiral DHP as well as progress in the pharmacology of the Ca channel.

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