The $p$H-controlled antiarrhythmic action of dihydropyridines

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Investigations on the $ab$ initio Hartree Fock molecular orbital calculations on Ca$^{2+}$ channel blockers, in conjunction with intermolecular interaction calculations are reported here. The effect of substitution in the phenyl/pyridyl ring on the activity of the compound is discussed. The conformational mapping clearly indicates the differences in the disposition of phenyl ring with respect to the pyridyl ring. These differences lead to significant differences in terms of interactions with the Ca$^{2+}$ ion and are able to explain the differences in the activity of $\omega$, $m$-, and $p$-nifedipine. The Ca$^{2+}$ ion holding capacity of the drugs has been investigated by drug-ion interaction energy calculations. The results indicate that the unprotonated form of the drug is capable of blocking the channel by holding the ion on the essential side of the drug, while the protonated form is not capable of holding the Ca$^{2+}$ ion. Depending on the state of protonation/unprotonation, a possible mechanism explaining use dependent blockade by DHP’s has been suggested. The change in $p$H could result in change in state of protonation and thus on/off interactions with the Ca$^{2+}$ ion.

Ions play a vital role in a large number of cellular processes including excitation-contraction and stimulus secretion$^1$. Voltage-gated Ca$^{2+}$ channels are transmembrane proteins which upon membrane depolarization allow selective Ca$^{2+}$ permeation mediating important cellular processes like muscle contraction, hormone secretion, etc. L-type Ca$^{2+}$ channels (LCC’s) are sensitive to numerous agonist and antagonist drugs that modulate Ca$^{2+}$ flow. Three major classes of compounds have been identified as LCC agonists or antagonists, i.e. LCC activators or blockers$^2$. 1,4 Dihydropyridines (DHP’s) form the major class of antiarrhythmic drugs including both activators and blockers of the channel; activation/blockade being probably determined by phenyl-pyridyl conformation$^3$. DHP’s being smaller in size, theoretical calculations have been done apart from regular molecular pharmacology studies. DHP’s including both activators and blockers have also been the center of interest. Most of the DHP’s exist in unprotonated form; their $p$Ka’s being very low at physiological $p$H$^4$.

The present investigations deal with the mechanistic aspects of antiarrhythmic action of DHP’s. We have already reported our $ab$ initio HF/6-31G molecular orbital calculations (M.O.) in detail on the conformational aspects of DHP’s. The effect of substituents in the pyridyl/phenyl ring and effect of protonation on the conformation and activity of dihydropyridines has been studied. Hartree Fock (HF) $ab$ initio MO calculations using a reasonable size basis set are nowadays completely feasible on such systems. We have chosen high potency representatives from the dihydropyridine class of compounds identified as Ca$^{2+}$ channel blockers (Str. I, II and III).
A quantum pharmacological procedure has been carried out (as explained in our previous work)\(^5\) that enables us to extract common pharmacophoric features. The conformational aspects have been studied in detail. In our earlier work, the effect of substituent at \(\alpha-, \beta-, \gamma-, \text{and } \delta\)-positions in the phenyl ring was studied\(^6\). The effect of having bulky ester group at 3,5 positions in the pyridyl ring was also studied. This study discussed the implications of these conformational aspects on the activity of the drug. The electrostatic aspects, i.e., the charge environment of the drugs studied through molecular electrostatic potential maps was also reported in our previous work on Ca\(^{2+}\) channel blockers. In the present study, the capability of the drug to obstruct the flow of ions in the channel by holding the ion has been studied through intermolecular interactions with the Ca\(^{2+}\) ion. The drug does not covalently bind the Ca\(^{2+}\) ion but temporarily holds it by nonbonded intermolecular interactions. We have also investigated drug’s affinity to anchor to a DHP sensing residue inside the channel in unprotonated/protonated form. The mechanistic implications of these results have also been discussed.

**Methodology**

*Ab initio* Hartree Fock (HF) molecular orbital (MO) calculations have been performed on Ca\(^{2+}\) channel blockers using 6-31G basis set\(^7\). Complete geometry optimizations have been performed for all using an optimally conditioned method of Davidson and Nazareth\(^8,9\). Complete conformational phase space has been scanned in each case. The starting point of DHP’s is the bioactive syn-anti conformation. Optimized conformations have been plotted with the help of ORTEP package\(^1\). To understand the differences in the conformation of various DHP’s, the drugs have been mapped one by one onto nifedipine considered to be prototype for DHP’s. This is referred to as conformational mapping. The idea of conformational mapping is to understand the variations in the conformation of the drug with respect to a clinically tested potent drug (which can be assumed to bind perfectly). The relative capacity of the drugs to hold the Ca\(^{2+}\) ion has been investigated by calculating intermolecular interaction energies at the 3-21G level using a supermolecule approach.

\[ \Delta E_{\text{int}} = E_{\text{AB}}^\text{Supermol} - (E_A + E_B) \]

For intermolecular interaction calculations, the initial geometry of the complex is taken from geometry optimized at the 6-31G level for the drug. Ca\(^{2+}\) ion position is now optimized at the 3-21G level as higher basis set is not available for Ca\(^{2+}\). As far as mechanistic aspects are concerned, we are only interested in relative intermolecular interaction energies. The amount of error due to choice of basis set would consistenly remain the same and hence cancel out when looking at relative interaction energies. While scanning on the distance and angles of approach of Ca\(^{2+}\), the scan is stopped if Ca\(^{2+}\) comes within covalent distance from the atom towards which it is approaching. We avoid the word ‘chelation’ as it typically means coordinate bond between the ligand and the ion. The geometry of the complex is thus optimized, with this constraint that the Ca\(^{2+}\) ion should not be covalently bound (i.e., the drug can hold the ion and also release the ion if needed, in other words, use dependent blockade\(^5\)). ‘Use-dependent Blockade’ theory assumes that the channel blocker drug binds the channel (Ca\(^{2+}\) ion) with varying affinity that leads to transition between different states, i.e., Resting, Open or Inactive states. In the same manner, drug’s affinity to H-bond to a DHP sensing residue Thr (I1S5.14) in the channel has been investigated. Again the DHP……Thr complex has been optimized considering the best possible H-bond with threonine in the ionized form. The results of these intermolecular interaction energy calculations have been analysed with respect to mechanistic aspects of DHP’s. The above calculations comprise the complete quantum pharmacological procedure.

**Results and Discussion**

The ORTEP plots of completely optimized geometries of studied calcium channel blockers at the HF level with the 6-31G basis set are shown in Fig. 1. The bioactive syn-anti conformations (disposition of 3 and 5 substituents with respect to pyridyl double bonds) have been studied. Although the syn-syn conformation is energetically more stable, syn-anti has been suggested to be the bioactive based on X-ray crystallographic studies and NMR studies\(^1\). However, there is no X-ray data for channel bound drug or drug obstructing Ca\(^{2+}\) flow. In both the conformations, the phenyl ring almost bisects the pyridyl ring in conformity with earlier studies. The nitro substituent on phenyl ring is in the \(\alpha\)-position in nifedipine. We have also optimized nifedipine where the substituents are in \(\alpha\)- to \(\beta\)- to \(\delta\)-positions. Moving the substituent from \(\alpha\)- to \(\beta\)- to \(\delta\)-positions does not seem to have much effect either on the disposition of the phenyl ring or on the coplanarity of C4 of pyridyl ring.
Angle between the phenyl and pyridyl ring in \( o \) is 110.88, \( m \) is 111.23, \( p \) is 110.80, \( C4-C5-C6-N1 \) in \( o \) =3.3, \( m \) =6.1, \( p \) =7.1. Earlier studies have indicated that the more active compounds exhibit smallest degree of ring distortion from planarity\(^1\). This is in conformity with our results as \( o \)-nitro nifedipine is known to be more potent as compared to \( m \)-nitro and \( p \)-nitro nifedipine and also shows least ring distortion from planarity\(^1\).

Figure 2, showing conformational mapping helps understand these findings and understand the effect of bulkier substituent at 3,5 positions in the pyridyl ring. Nisoldipine almost perfectly maps onto nifedipine. This shows the negligible effect of bulky substituent at 3/5 positions on C4 of pyridyl ring. In our earlier reported work on HIV-1RT inhibitors, we have seen that minor differences in drug conformation could lead to significant differences in drug-receptor interaction energies (when evaluated at the microscopic level) and hence explain their relative potencies\(^5\). Similarly, here minor differences in phenyl ring dispositions could lead to significant differences in their interaction energies with the \( Ca^{2+} \) ion and therefore different potencies.

We have also studied the effect of protonation on the conformation of the \( Ca^{2+} \) channel blockers. The effect of protonation can be best understood by mapping the protonated form onto the unprotonated form of the drug (Fig. 2). As expected, the major effect of protonation in DHP’s is on the disposition of phenyl ring with respect to pyridyl ring. Protonation of N decreases the coplanarity of C4. Our earlier work indicated an overall negatively charged environment on the DHP’s.

Overall charge environment was observed to be the same on both bioactive/bioinactive conformers. The difference in activity of various DHP’s was therefore discussed in terms of conformational aspects leading to significant difference in interaction energies with the \( Ca^{2+} \) ion when evaluated at the microscopic level.

To get a quantitative estimate for blockage of channel by unprotonated form of drugs we have evaluated interaction energies with the \( Ca^{2+} \) ion (shown in Fig. 3). \( Ca^{2+} \) ion has been allowed to approach the drug from the essential side\(^7\). The position of \( Ca^{2+} \) ion has been optimized to get minimum interaction energy (i.e. most attractive interaction with the drug); the only constraint being that the ion should not get covalently bonded to the drug with the idea that the drug only obstructs the flow of ions, it does not permanently hold the ion. In this way we are probing the blockage of \( Ca^{2+} \) channels by the unprotonated form of DHP’s. Figure 3 shows the calculated intermolecular interaction energies. It is
Fig. 2—Conformational mapping. [(a) m-nitro nifedipine mapped onto nifedipine; (b) p-nitro nifedipine mapped onto nifedipine; (c) nisoldipine mapped onto nifedipine; (d) unsymmetrical dihydropyridine mapped onto nifedipine; (e) protonated nifedipine mapped onto unprotonated nifedipine, and; (f) protonated nisoldipine mapped onto unprotonated nisoldipine].
clear from the figure that the most favorable position for Ca\(^{2+}\) ion is when it can interact with both, the substituents on phenyl ring as well as the ester substituent on 5-position in the pyridyl ring. When the substituent is moved to m- and p-positions in phenyl ring and simultaneously the non-coplanarity of C4 increases, interaction with the Ca\(^{2+}\) ion is reduced. In other words, the blocking capacity of the drug would be reduced. Nisoldipine shows similar interaction energy with the Ca\(^{2+}\) ion as o-nifedipine (the only structural difference in nisoldipine and nifedipine is the 3 ester substituent on pyridyl ring). IC\(_{50}\) values for various DHP's are also very similar. In our previous work on nucleoside inhibitors for HIV-1RT, we have shown correlation between the drug-receptor interaction energies and IC\(_{50}\) values. In the present case IC\(_{50}\) values for all DHP's being quite similar it can only be shown that interaction energies are also quite similar. Unsymmetrical DHP shows less attractive interaction with the ion, as there is no major interaction with the substituent on the 5-membered rings. We have also performed intermolecular interaction calculations on protonated nifedipine and Ca\(^{2+}\) ion. (Best interaction energy between protonated o-nifedipine...Ca\(^{2+}\) was observed to be 40.24 kCal/mol). As expected, it shows repulsive interaction, i.e. the protonated form of DHP's would not be capable of blocking the flow of Ca\(^{2+}\) ions in the channel.

We have also investigated the drug's ability to H-bond to a DHP sensing residue at the receptor site for DHP. Out of 6 DHP sensing residue Thr (III5.14) has been shown to be important in anchoring the drug to the active site. Figure 4 demonstrates the binding of the drug to the ionized threonine via H-bond formation. The figure demonstrates that the drug continues to remain bound to the receptor even when the proton has actually been transferred to the drug. Figure 4 also indicates that intact the drug is even
Fig. 4—DHP's affinity to form H-bond with Thr (HIS5.14) in the channel in unprotonated/protonated form. Threonine has been taken in the ionized form. Interaction energies are given within parentheses in kcal/mol. [(a) unprotonated nifedipine...Thr (–141.18); (b) protonated nifedipine...Thr (–184.73); (c) unprotonated nisoldipine...Thr (–142.38); (d) protonated nisoldipine...Thr (–222.16); (e) unprotonated unsymmetrical dihydropyridine...Thr (–138.17), and, (f) protonated unsymmetrical dihydropyridine...Thr (–119.04)].

Fig. 5—pH-controlled antiarrhythmic action of 1,4 dihydropyridines. [(a) unprotonated nifedipine hydrogen bonded to Thr and holding Ca$^{2+}$ ion in the channel, and, (b) protonated nifedipine hydrogen bonded to Thr and free Ca$^{2+}$ ion in the channel].
more tightly bound to the receptor, so that the Ca$^{2+}$ ions do not compete for the binding site and do not interfere as competitors at the active site, otherwise the whole mechanism will fail.

Based on these intermolecular interaction results, we now discuss implications to mechanistic aspects. Dihydropyridines when taken in as drugs exist in the unprotonated form in the body (at physiological pH). They bind to the receptor via H-bonding to one of the residues capable of proton donation. While binding to the receptor from the N-end of the dihydropyridine ring it is also capable of holding the Ca$^{2+}$ ions from its essential side (port side) as indicated by Fig. 5. Changes in physiological pH may induce protonation of the drug and the protonated form of the drug is not capable of holding the Ca$^{2+}$ ions as indicated by our intermolecular interaction calculations. Hence, a self-regulatory pH-controlled mechanism may be thought of as a use-dependent blockade in DHP's.

Conclusions
The substitution in the phenyl ring in DHP's does not have significant effect on the disposition of the phenyl ring with respect to the pyridyl ring, but these minor differences lead to significant differences in interaction energies (when evaluated at the microscopic level) with the Ca$^{2+}$ ion so as to explain the decreased activity of $p$-nifedipine as compared to $o$- and m-nifedipine. 3/5 bulky substituents in the pyridyl ring and their disposition with respect to pyridyl double bonds has minor effects on the coplanarity of C4 as indicated by our conformational mapping.

The optimal approach of Ca$^{2+}$ ions from the essential side of the drug depends on conformational as well as electrostatic aspects; most favourable position being attractive interactions with the pyridyl and phenyl substituents. Protonated form of the drug would probably be anchored to the nearest site on the channel whereas the unprotonated form would block the channel by holding the Ca$^{2+}$ ions as indicated by our interaction energy calculations.

Depending on the state of protonation of the drug at physiological pH, there is a possibility of explaining the use dependent blockade by DHP's. Change in pH could result in protonation/unprotonation of the drug and hence off/on interactions with the Ca$^{2+}$ ion in DHP's.

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