PHENYLALKYLAMINES AS Ca\textsuperscript{2+} CHANNEL BLOCKERS

Phenylalkylamines (PAA) block voltage-gated Ca\textsuperscript{2+} channels, by binding within the intracellular mouth of the ion conducting pore [1]. Block of L-type Ca\textsuperscript{2+} channels in cardiac and smooth muscle by Verapamil and related PAA's is an important therapy for hypertension, cardiac arrhythmias and angina pectoris [2].

7.1 Previous Conformational Studies on Phenylalkylamines:

Verapamil belongs to the Phenylalkylamine group of calcium channel antagonists and has been extensively used clinically in the treatment of several cardiovascular diseases [3]. S. Tetrault et al. [4] suggest that Verapamil adopts a relatively compact structure. They conducted molecular simulations for the free drug with the crystal structure of verapamil as the starting point [5]. In the computed solution structure the two phenyl rings are much farther apart from each other than in the crystal, so that the drug molecules appear to
be "open" in solution. The relative orientation of the phenyl rings with respect to each other is however, similar in both the structures (angle between the ring planes is \( \approx 90^\circ \) in solution and \( \approx 70^\circ \) in the crystal) [5]. Gaggeli and coworkers [6] also studied the solution structures of Verapamil in deuterated DMSO using one-dimensional \(^1\text{H}\) and \(^{13}\text{C}\)-NMR data, which led to a preferred conformation for the drug where both the phenyl rings are out of the plane of the aliphatic backbone.

Another drug of PAA subclass is gallopamil (D600); also widely used in clinical medicine and experimental biology to block \(\text{Ca}^{2+}\) inflow into cell across the plasma membrane [7]. Brausseur et al. [8] studied the conformational features of gallopamil and gave the analysis that the neutral form of the drug is characterized by a unique conformation; whereas the protonated form exist in different conformation with great mobility of the torsional angles and of the ionized site of the molecule. The capacity of gallopamil to inhibit ionophore-mediated calcium translocation in a two-phase bulk system was inversely related to the pH of the aqueous phase. These findings indicate that the capacity of gallopamil to interfere with the
transport of cations is critically dependent on the availability of a protonated configuration of the drug.

Three molecules within the PAA class have been extensively studied: Verapamil, D888 and Methoxyverapamil. D888 contains only one meta methoxy group at the inside of the aromatic of the phenylethyl part, whereas Verapamil contains two methoxy groups in meta and para positions. D888 blocks the channel with higher affinity than Verapamil (~300 fold) [9], although Verapamil is the only drug in the class currently in clinical use. The levo rotatory (-) enantiomers of PAAs are more potent than the dextrorotatory (+) enantiomers [10]. Verapamil can adopt three structural forms (or conformational shapes): extended, folded and half-folded [11]. The folded conformation is stabilized by nonbonded interactions between the two dimethoxy aryl rings situated on the opposite ends of Verapamil and is suggested to be the most stable conformation of the isolated drug.

7.2 Receptor Binding and Antagonistic Activity of Phenylalkylamines:
Binding sites for all three classes of drugs are located below the channel’s selectivity filter. Indeed quaternary amine derivative of PAA (Verapamil) gain access to their binding sites from the cytoplasm [12] and seem to inhibit the central pore by physical occupancy. The PAA binding pocket is composed of at least seven amino acid residues [13], based on the alanine scanning mutagenesis of binding of the PAA derivative desmethoxyverapamil (D888). Three amino acid residues in segment IVS6- Tyr1463, Ala 1467 and Ile 1470 - are required for high affinity block by D888, because their mutations reduced the affinity 6-12 fold. However Hering et al. [14] also included Met 1464 as a residue contributing to high affinity PAA interaction.

Verapamil has been reported to inhibit \[^3\text{H}\]Diltiazem binding in a non-competitive manner through a negative allosteric interaction, although Verapamil apparently mimics competitive interaction [15]. Electrophysiological data have indicated that the binding domain for PAA is located on the intracellular side of the membrane in cardiac myocyte [16].
V.S. Ananthanaryanan showed that in the Ca$^{2+}$-bound form, two Verapamil molecules are arranged with a 2-fold symmetry such that the two methoxy oxygens from each molecule act as ligand to the cation [17]. The case for Ca$^{2+}$ as necessary factor modulating Verapamil's binding to its receptor is quite strong. Although early studies indicated an inhibitory effect of Ca$^{2+}$ on the binding of PAAs to the receptor; there is also evidence that suggests, there could be a concentration dependence of the Ca$^{2+}$ effect [18]. Low Ca$^{2+}$ concentration seems to favor binding while an excess of Ca$^{2+}$ lowers the affinity of the channel for PAA. Data using a new fluorescent probe that interacts at the PAA site reveal that removal of Ca$^{2+}$ results in a lowering of the binding affinity for these drugs [19].

DHPs' show almost exclusively tonic block, while PAAs show solely use-dependent block and BTZ are intermediate [20]. This specific property may be because of huge conformational change required. PAAs also exist in the protonated form at physiological pH and their pKa have been reported to be 9.04 [21].

IC$_{50}$ value of PAAs in µM is 12 ± 5 (mean ± S.E. of four cells) [15].
7.3 Our Results and Discussion:

7.3.1 Conformational and Electrostatic Aspects:

First of all, we have located the global minimum on the potential energy surface to be taken as the bioactive conformation in absence of information on actual bioactive conformation. The conformations corresponding to located global minima for the unprotonated and protonated form of PAA are shown in Fig. 25. The unprotonated form occurs in the "sandwich" or folded conformation and the protonated in the half folded form. Protonation is obviously done at the amine N. There is no other site available for protonation in PAA’s. The next step was to perform the conformational mapping.

Conformational mapping clearly indicates huge conformational change on protonation of drug (Fig. 26). Fig. 25 shows that in both the conformations (unprotonated/folded; protonated/half folded) the aryl rings are out of plane with respect to the aliphatic backbone. In the half folded form the two aryl rings are at an angle of 71.50°. The unprotonated folded/compact form is similar to the conformation observed in the crystal; angle between the ring planes is predicted to be 80.85°. It is interacting to note that both the forms are ‘R’ enantiomer with respect to chiral center.
Fig. 25 ORTEP drawings of optimized bioactive conformations of protonated and unprotonated Verapamil
Earlier studies have shown some importance of chiral center with reference to the activity of the system. Another important pharmacophoric features is the distance between the two aryl rings which is predicted to be 8.5 Å in the half folded, protonated form and reduced to only 5.7 Å in the folded form on deprotonation.

The charge environment of the drug has been studied utilizing calculation of molecular electrostatic potential. The calculation of complete molecular electrostatic potential maps indicates an overall negatively charged environment on PAA (Fig. 27) in unprotonated form. However, as the drug enters the body in protonated form it may be anchored to the receptor via H-bond formation involving protonated amine.

7.3.2 Ca$^{2+}$ Ion Holding Capacity:

We now discuss our intermolecular interaction calculations to understand the Ca$^{2+}$ ion holding capacity of PAA’s. In the case of PAA after several tries attractive interaction was found only in single case, where the Ca$^{2+}$ ion electrostatically interacts with the methoxy groups on aryl ring close to the chiral carbon (Fig. 28).
Unprotonated Verapamil mapped onto protonated Verapamil

Fig. 26 Conformational Mapping

MESP for unprotonated Verapamil

Fig. 27 MESP contours for unprotonated Verapamil. Red coloured contours indicate a value of -0.1 for electrostatic potential and yellow contours indicate a value of -0.05 e.s.u.
Fig. 28 Optimized unprotonated Verapamil...Ca$^{2+}$ Complex

Int. En. = -93.56 kCal/mol
Ca$^{2+}$ ion in its most preferable position can be seen approaching from above the aryl ring, so as to avoid steric interactions and be able to electrostatically interact with cyanide group and the two methoxy substituents on the aryl ring.

7.4 Conclusions:

1. The most favorable conformation based on gas phase calculations for unprotonated form of Verapamil is the folded structure.

2. Protonated form is predicted to be in half folded/unfolded conformation.

3. As the drug enters the body in protonated form it is expected to be anchored to the receptor via H bond formation involving protonated amine of PAA.

4. The pharmacophoric features extracted from optimized conformation predict that the two dimethoxy aryl groups should be disposed at an angle 80.85° in the unprotonated/folded form.

5. Both phenyl rings are out of plane with respect to aliphatic backbone.

6. Folded, unprotonated form is capable of holding the Ca$^{2+}$ ion. Chiral centre also seems to be involved in channel blocking in conformity
with previous studies showing different potencies for different enantiomers.
References:


   


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