Interaction of Calcium Channel Antagonists with Calcium: Structural Studies on Nicardipine and Its Ca^{2+} Complex

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Conformational features of nicardipine in acetonitrile, in the absence and presence of Ca^{2+}, were investigated by one-dimensional NMR and difference absorption spectroscopy techniques. The data show that in acetonitrile solution the *antiperiplanar* form of nicardipine is dominant. The addition of Ca^{2+} to the drug solution caused marked changes in the difference absorbance spectra in the 200–400 nm region and in many of its ^1H and ^{13}C NMR resonances. The changes were most significant up to a ratio of 0.5 Ca^{2+}:drug. Analysis of the binding data showed the predominant species to be a 2:1 drug:Ca^{2+} "sandwich" complex with an estimated dissociation constant of 100 μM at 25 °C. One-dimensional nuclear Overhauser effect (NOE) experiments revealed through-space connectivities in the drug before and after Ca^{2+} binding. These changes in conjunction with the changes in ^1H and ^{13}C chemical shifts suggest a structure in which the 4-aryl ring substituent of the pyridine moiety moves closer to the C3-side chain in the presence of Ca^{2+}. This attraction is achieved *via* the chelation of the Ca^{2+} ion by the oxygen atoms in the *m*-NO_2 of the aryl group and the $COOCH_2$ group in the side chain of the dihydropyridine ring, and gives rise to a stable *synperiplanar* conformation. A preference for this conformation was also observed in the Ca^{2+} complex of nifedipine in acetonitrile as inferred from the rather limited NOE data obtained. Our study provides a detailed solution structure for nicardipine and also leads to a suggestion of a role for Ca^{2+} in the action of this and possibly other dihydropyridines.

Introduction

Calcium channel blockers are well-known cardiovascular drugs used clinically in the treatment of angina pectoris and hypertension.[1] These drugs inhibit the voltage-gated calcium channels in smooth muscle and cardiac muscle cells. An astonishing variety of chemical structures are found among the calcium channel blocking drugs.[2] Phenylalkylamines, benzothiazepines, and 1,4-dihydropyridines (DHPs) are three major classes of blockers and are represented by verapamil, diltiazem, and nifedipine, respectively. Due to their diverse chemical structures and biological activities, these compounds have become attractive targets for studies on molecular mechanism of action and on conformational features.[2] A common functional attribute shared by the above three classes of drugs is their binding to the DHP-sensitive receptor where they appear to interact in an allosteric fashion.[3] While significant advances in the study of the structure of the DHP-sensitive receptor have recently been made[4–6] and proposals about the drug-binding sites have been advanced,[7] knowledge of the bioactive conformation of the drugs at the sites of their interaction with the respective receptors is limited.[2a,2b] We have sought to approach this problem in terms of the plausible interaction of drugs with extracellular Ca^{2+} in the vicinity of the lipid bilayer membrane. This is based on the suggestion[8] that the Ca^{2+}-bound form of these molecules may represent their bioactive conformations. In the case of the calcium channel antagonists, a number of recent studies have pointed out the Ca^{2+} dependence of the drugs' binding to the calcium channels.[9] Recent publications from our laboratory have demonstrated that verapamil[10] and diltiazem[11] bind Ca^{2+} in low-dielectric media giving rise to both 2:1 ("sandwich") and 1:1 drug:Ca^{2+} complexes.[12] Similar data on DHP drugs are not available. They would, however, be valuable, particularly since a large body of information is available on the DHP-sensitive L-type calcium channel.[3,7] This channel also contains binding sites for the other types of calcium channel antagonists,[3] and hence it would be interesting to know if a common requirement for drug binding exists that requires the prior formation of drug:Ca^{2+} complex. With this in mind, we have extended our studies to nicardipine [1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl 2-[methyl(phenylmethyl)amino]ethyl ester], a DHP representative, in order to determine the nature of its interaction with Ca^{2+}. The conformations of free and Ca^{2+}-bound nicardipine were derived from spectroscopic data, particularly nuclear Overhauser effect (NOE) difference spectroscopy.

Experimental Section

Materials. Nicardipine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO), deuterated acetonitrile (CD_3CN) from MSD Isotopes (Quebec, Canada), acetonitrile (CH_3CN) from Fisher Scientific (Ontario, Canada), and calcium perchlorate [$Ca(ClO_4)_2$] from VWR Scientific (Ontario, Canada). CH_3CN was predried over molecular sieves, distilled and stored over molecular sieves (4 Å), and kept under argon. $Ca(ClO_4)_2$ was lyophilized overnight to remove any trace of water.

Methods. UV Spectrophotometry. UV spectra were recorded using a Perkin-Elmer Lambda 6 spectrophotometer equipped with a microprocessor for spectral collection and data manipulation. Spectra were recorded at room temperature (25 ± 1 °C) in dry CH_3CN using a quartz cell of 1 cm pathlength.

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Drug concentration used was 180 μM as determined from dry weight. Difference absorption spectroscopy was performed by adding small aliquots of $Ca(ClO_4)_2$ in CH_3CN, with mixing, to a solution of nicardipine (in the same solvent) in the sample cuvette and corresponding aliquots of CH_3CN to the reference cuvette followed by recording of the difference spectrum, between 200 and 400 nm, after each addition.

NMR Spectroscopy. ^1H and ^{13}C NMR spectra were recorded on Bruker AC-300 and AC-500 spectrometers at a probe temperature of 30 °C. CH_3CN was used as an internal reference for both ^1H (1.93 ppm) and ^{13}C (1.3 ppm) NMR spectra. The ^{13}C resonances were assigned using the heteronuclear (^1H-^{13}C) shift-correlation method (XHCORR) at 30 °C. The drug concentration used was 10 and 20 mM in CD_3CN for ^1H and ^{13}C experiments, respectively. ^1H spectral line widths did not show significant changes in the concentration range 5–20 mM, implying that aggregation of the drug is negligible. Titration of the drug with Ca^{2+} was done by adding, with mixing, aliquots of a stock solution of $Ca(ClO_4)_2$ in CD_3CN to the drug solution inside the 5-mm NMR tube to obtain the desired drug:Ca^{2+} mole ratios. Dilution of the sample by Ca^{2+} addition was less than 5%. One-dimensional NOE difference spectra on nicardipine and its Ca^{2+} complex were recorded using the 500 MHz instrument by subtracting an off-resonance control spectrum from the individual spectra obtained by selective irradiation of each proton signal. A relaxation delay of 5 s for both the control and on-resonance free induction decays (FIDs) was used. The decoupler was gated off during acquisition. Eight scans were acquired for both the control and on-resonance FIDs. A cycle of alternate acquisition of the above-mentioned FIDs was repeated four times for a total of 32 scans for the final difference FID. A 90° ^1H pulse width of 18.6 μs was used.

Results

Calcium Binding by Nicardipine: Absorption Spectroscopy. Difference absorption spectroscopy in the 200–400 nm region was used in order to determine whether any interactions between the drug and the metal ion take place. Nonzero difference spectra saturating at about 0.5 Ca^{2+} mole ratio were obtained with a positive peak around 375 nm and two negative peaks around 270 and 320 nm. This behavior indicates that Ca^{2+} interacts with nicardipine and induces a change in the environment of the DHP (absorption maximum: 350 nm) and benzene rings (absorption maximum: 268 nm) in the drug. The change in the difference absorbance was used to construct the binding isotherm at 25 ± 1 °C, and the curve obtained is shown in Figure 1. As was done earlier for verapamil[10] and diltiazem,[11] an analysis of the binding curve was performed using the method of Reuben[13] in order to assess the nature of the complexes formed and to estimate the dissociation constants for the 1:1 and 2:1 drug:Ca^{2+} complexes. The values obtained for K_1 and K_2 are 100 and 26 μM, respectively, suggesting that the 2:1 drug:Ca^{2+} complex is favored (Figure 2).

^1H and ^{13}C NMR Studies. Figure 3 shows the numbering schemes used in ^1H and ^{13}C NMR assignments of the nicardipine and nifedipine resonances. Most of the ^1H resonances in nicardipine were assigned by comparison with the reported chemical shift data on nifedipine.[14] The chemical shifts for protons H_A, H_B, H_D, H_E, and $COOCH_2$ in the side chain attached to C3 were determined by using Shoolery's rules which permit the calculation of a shift position of a methylene group attached to two functional groups.[15] Proton H_C is not observed probably due to exchange processes.

The ^1H chemical shift data for nicardipine in CD_3CN are listed in Table 1. The ^{13}C resonances were assigned unambiguously by 2D ^1H–^{13}C correlation spectroscopy, and only the peaks of interest are shown in Table 2. As far as the structure of the neat drug is concerned the

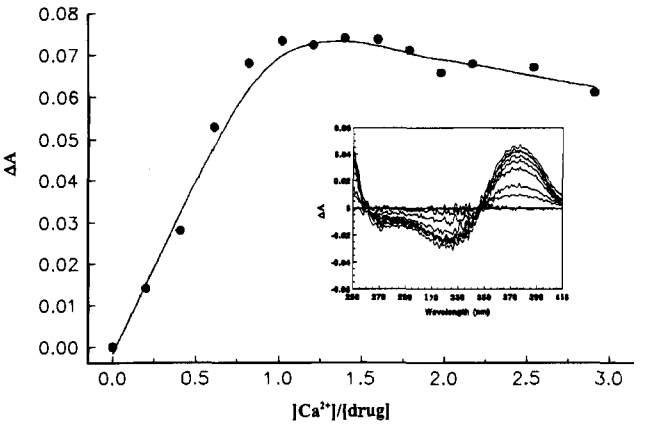


Figure 1. Plot of the difference molar absorbance (ΔA) of nicardipine in CH_3CN at 348 nm as a function of Ca^{2+} concentration at 25 ± 1 °C. Inset: Difference spectra of nicardipine produced by addition of $Ca(ClO_4)_2$ in CH_3CN at 25 ± 1 °C. Ca^{2+} concentration increases from zero (bottom curve) to 1.8 molar equiv (top curve) in increments of 0.2 molar equiv of the cation.

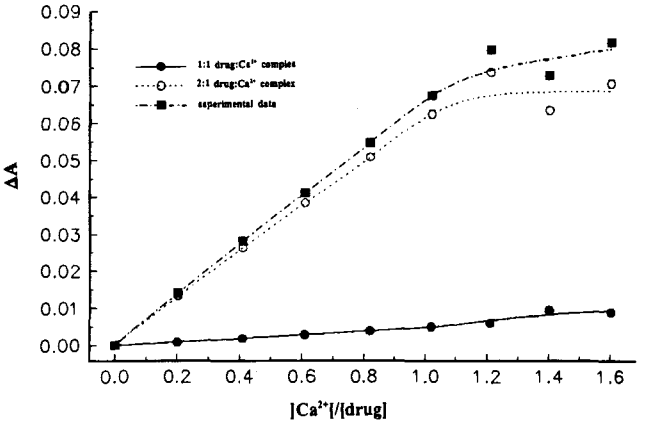
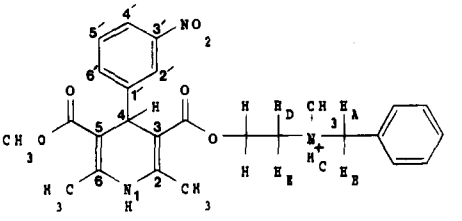
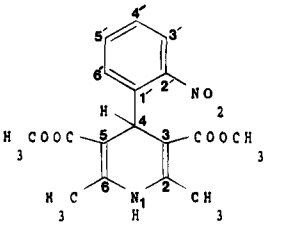


Figure 2. Ca^{2+} binding curves for nicardipine at 25 °C obtained from difference absorbance changes at 375 nm (ΔA). The method of Reuben[13] was used to obtain the computed curves.



Nicardipine



Nifedipine

Figure 3. Molecular formulas and numbering schemes for nicardipine and nifedipine.

Table 1. ^1H Chemical Shifts of Nicardipine and Its Calcium Complex in CD_3CN at 30 °C

proton	chemical shift (ppm)	
	free drug	$[Ca^{2+}]/[drug] = 1.5$
C2-CH_3, C6-CH_3	2.26, 2.33	2.28, 2.30
NCH_3	2.49	2.72
H_D	3.26	3.31
H_E	3.15	3.31
$COOCH_3$	3.58	3.6
H_A	4.15	4.35
H_B	3.97	4.28
$COOCH_2$	4.47	4.37
C4-H	5.05	5.03
C_6H_5	7.4	7.43
H-6′	7.58	7.47
H-5′	7.64	7.61
H-4′	7.91	7.96
H-2′	8.03	8.02
NH		7.34

Table 2. ^{13}C Chemical Shifts of Nicardipine and Its Calcium Complex in CD_3CN at 30 °C

carbon	chemical shift (ppm)	
	free drug	$[Ca^{2+}]/[drug] = 1.5$
C2-CH_3	18.81	18.79
C6-CH_3	19.11	19.21
C4	40.28	40.31
N-CH_3	40.28	41.38
CH_3O	51.47	51.58
OCH_2	54.67	56.35
CH_2N	59.06	58.91
CH_2Ph	60.28	61.34
C2	101.41	101.47
C6	103.35	103.64
$COOCH_2$	167.36	168.45
$COOCH_3$	168.4	168.45

following observations can be made from the ^1H spectrum in Figure 4A. The methyl protons, C2-CH_3 and C6-CH_3, are nonequivalent, thus giving rise to two singlets. The methylene protons H_A, H_B, and H_D, H_E are diastereotopic with values of $\delta_A - \delta_B = 0.18$ ppm and $\delta_D - \delta_E = 0.12$ ppm, respectively. Protons H_D and H_E give two broad multiplets which clearly indicate additional coupling from $COOCH_2$.

The effect of Ca^{2+} addition on the NMR of nicardipine is presented in Tables 1 and 2. Progressive addition of the cation causes significant changes in the ^1H and ^{13}C resonances in the drug (Figure 4B, and Tables 1 and 2). It is interesting to note that in the presence of excess Ca^{2+}, the methyl protons attached to C2 and C6 are still nonequivalent but with a much smaller chemical shift difference, $\Delta\delta_{CH3}$, of 0.02 ppm; $\Delta\delta_{CH3}$ in the neat drug is 0.07 ppm. In addition, the resonances for H_A, H_B, H_D, H_E, and $COOCH_2$ protons are broader when compared to the Ca^{2+}-free drug. The NH proton of the DHP moiety was observed only in the Ca^{2+}-bound form of the drug and resonates at 7.34 ppm; in the neat drug the NH proton probably overlaps with the C_6H_5 resonance.

The ^1H chemical shift change, $\Delta\delta$, as a function of the mole ratio of Ca^{2+} to drug is graphically presented in Figure 5 for several protons. In all cases $\Delta\delta$ increases up to a Ca^{2+}:drug molar ratio of about 0.6 and saturates at higher Ca^{2+}:drug ratios. Hence, the chemical shift changes in the ^1H and ^{13}C resonances are not due to changes in the bulk properties of the medium but are a result of the donor–acceptor interactions in the drug–metal ion system. The $\Delta\delta$s between the free and Ca^{2+}-bound drug are relatively large for NCH_3, H_D, H_B,

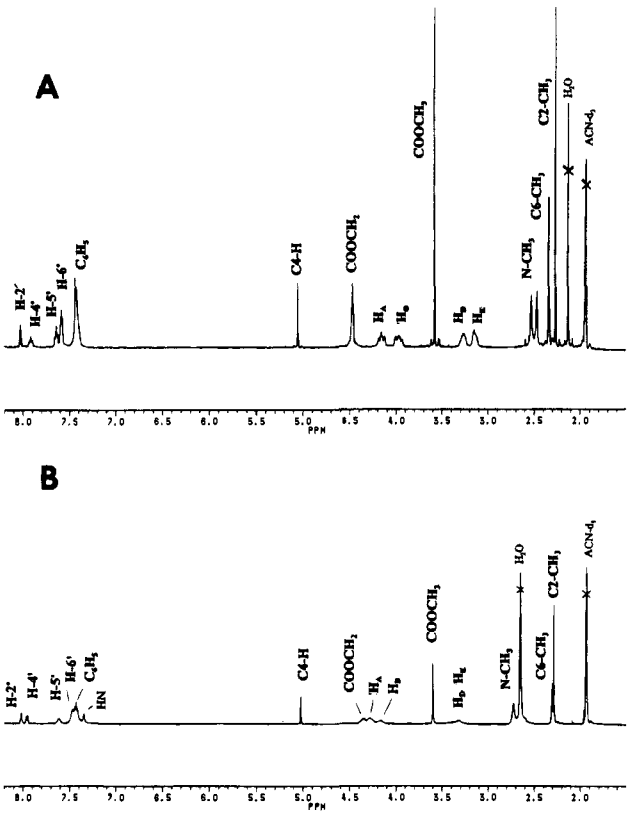


Figure 4. ^1H NMR spectra of nicardipine: (A) free drug, (B) drug + 1.5 molar equiv of Ca^{2+}.

$COOCH_2$, C4-H, and H-2′ (ranging from 0.11 ppm to 0.22 ppm). Smaller values are observed for C2-CH_3, C6-CH_3, $COOCH_3$, H_A, H_E, H-4′, H-5′, and H-6′ (ranging from 0.02 to 0.05 ppm) (Figure 5). The fact that the protons in the vicinity of the carbonyl group (situated in the side chain attached to C3) and the nitro group (in the C4-aryl moiety) exhibit significant induced shifts in the presence of Ca^{2+} is a clear indication of the chelation of the metal ion by these groups. It is interesting to note that the C5 carbonyl function is less affected by the Ca^{2+} ligand when compared to the C3 carbonyl as evident from the examination of the ^{13}C data in Table 2; thus $COOCH_3$ carbonyl shows a smaller ^{13}C chemical shift change ($\Delta\delta = 0.05$ ppm) when compared to $COOCH_2$ ($\Delta\delta = 1.1$ ppm). This suggests that the carbonyl group in the C3 side chain may be directly involved in chelating the metal ion. The Reuben[13] analysis of the binding curves obtained from the ^1H NMR results gave dissociation constants of about 300 μM for K_1 and 50 μM for K_2, which compare well with the difference spectroscopy data and suggest the dominance of a 2:1 drug:Ca^{2+} complex (Figure 6).

1D-NOE Studies. The observation of the nuclear Overhauser enhancements (NOEs) reflects the relative spatial disposition of various hydrogen atoms in the molecule and thus provides an insight into the three-dimensional structure in solution. The NOE decreases rapidly with interproton distance[16] and may therefore be used as an approximate measure of the proximity of two dipolar-coupled protons if they are less than 5 Å apart.[17a] Figure 7 illustrates the most significant NOE difference spectra obtained with nicardipine and its Ca^{2+}-bound complex.

It has been shown that DHPs occur either as the sprotamer, in which the substituent on the 4-aryl ring and

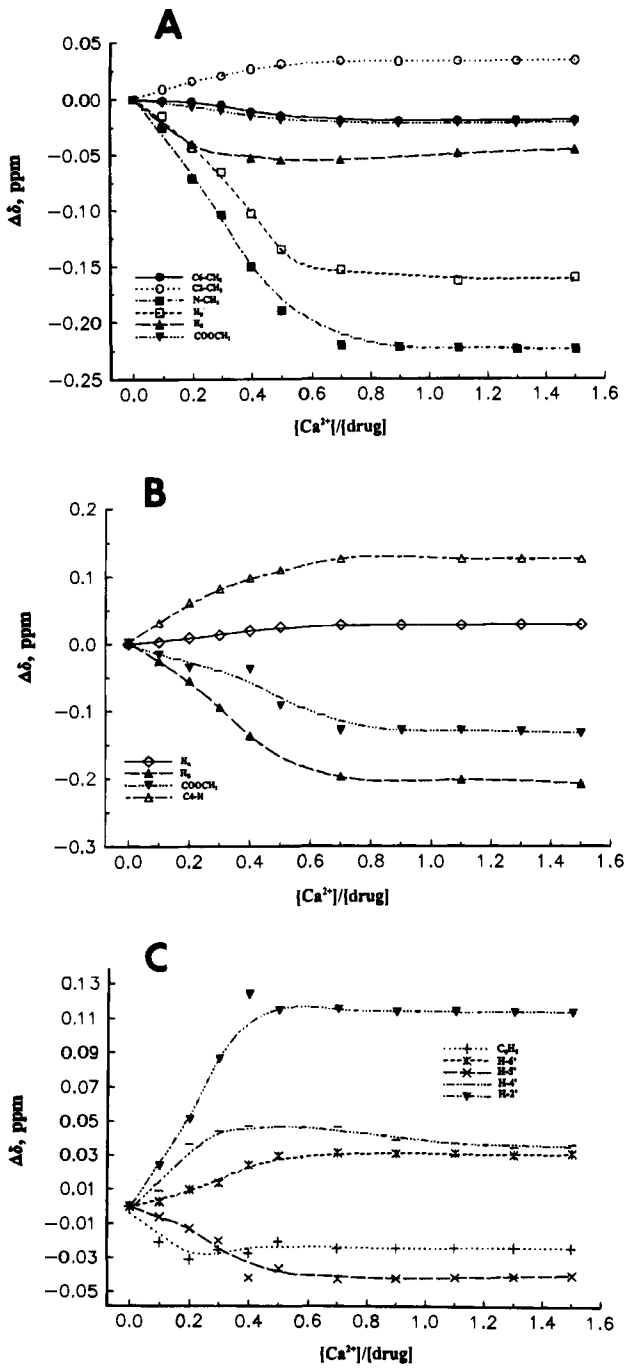


Figure 5. Plots of the Ca^{2+}-induced shifts of ^1H resonances in nicardipine as a function of $[Ca^{2+}]/[drug]$ mole ratio for the indicated protons.

the hydrogen atom at the 4-position are synperiplanar, or as the ap-rotamer, in which the same groups are antiperiplanar.[18] Decoupling of the methyl protons at 2.26 ppm (C2-CH_3) (Figure 7B) and 2.33 ppm (C6-CH_3) (Figure 7C) in the free drug establishes two structural features: firstly, the nonequivalence of these two groups as shown by the $COOCH_3$ enhancement observed only on decoupling of C6-CH_3 protons and secondly, the proximity of both methyl groups to the unsubstituted phenyl ring which exhibits an NOE on irradiation of either of the methyl protons (Figure 7B,C). This conformation, in which the phenyl ring approaches both methyls, results from the unhindered rotation around carbon–carbon single bonds in the ester side chain attached to C3. In contrast, irradiation of C2-CH_3 and

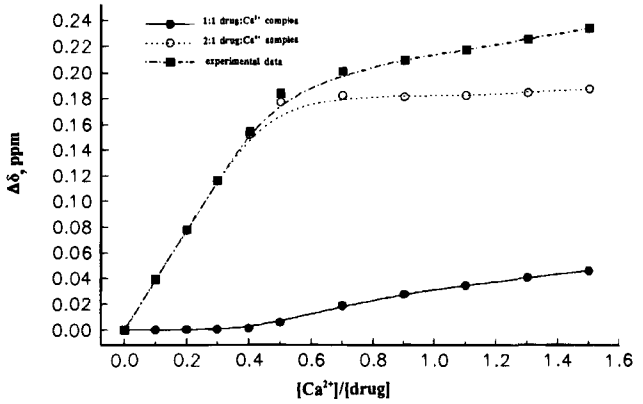


Figure 6. Ca^{2+} binding curve for nicardipine obtained from the NCH_3 proton chemical shift changes ($\Delta\delta$). The method of Reuben[13] was used to obtain the computed curves for the 1:1 and 2:1 complexes.

C6-CH_3 protons in the drug containing Ca^{2+} results in a positive enhancement of the NH proton (of the DHP ring) as well as in a small NOE from H-4′ (Figure 7J) with no observable NOE of the phenyl ring protons. This observation is consistent with a conformation in which Ca^{2+} chelates to the CO group in the C3 side chain and to the *m*-NO_2 substituent thus bringing H-4′ closer to the C2- and C6-CH_3 groups.

Decoupling of the NCH_3 protons (2.49 ppm) in the free drug results in small NOEs from protons in the C3 side chain (H_D, H_E, H_A, H_B, and $COOCH_2$), as well as H-4 and H-6′ protons (Figure 7D). Decoupling of the same protons in the drug containing Ca^{2+} enhances the C3 side chain resonances significantly. Furthermore, the emergence of an NOE from H-2′ proton and the disappearance of the H-6′ NOE are also observed[19] (Figure 7K). These data suggest that in both the free and Ca^{2+}-bound drug and C3 side chain folds in such a way as to bring the NCH_3 protons close to the C4-aryl ring. In addition, in the free drug, the C4-aryl ring adopts a conformation in which the *m*-NO_2 substituent is antiperiplanar with C4-H. This is inferred from the NOE of the H-6′ aromatic proton on irradiation of NCH_3 and from the absence of H-2′ enhancement. In the presence of Ca^{2+}, in contrast, H-2′ and NCH_3 protons in the drug are close to each other in space. Model building shows that this can result only from a conformation in which the *m*-NO_2 substituent is synperiplanar with C4-H and that the side chain is folded toward the DHP ring (Figure 8).

Irradiation of the diastereotopic H_D and H_E protons in the neat drug gives significant enhancement of $COOCH_2$ signal and small NOEs from H_A, H_B, NCH_3, H-5′, and H-6′ resonances (Figure 7E,F). In the Ca^{2+} complex, however, all the aromatic protons (H-2′, H-4′, H-5′, and H-6′) give significant NOEs, and H_A, H_B, and $COOCH_2$ resonances are much more enhanced when compared to the free drug (Figure 7L). This behavior lends further support to the observation made earlier that binding of Ca^{2+} ions brings the C3 side chain and the C4-aryl group closer in a relatively rigid conformation.

Decoupling of the $COOCH_2$ protons in the free drug gives rise to NOEs from protons H_D and H_E (Figure 7G). There is also some indication of NOE of the C_6H_5 ring protons (Figure 7G). In the Ca^{2+}-bound drug, however, positive NOEs from C_6H_5 and, additionally, from C4-H

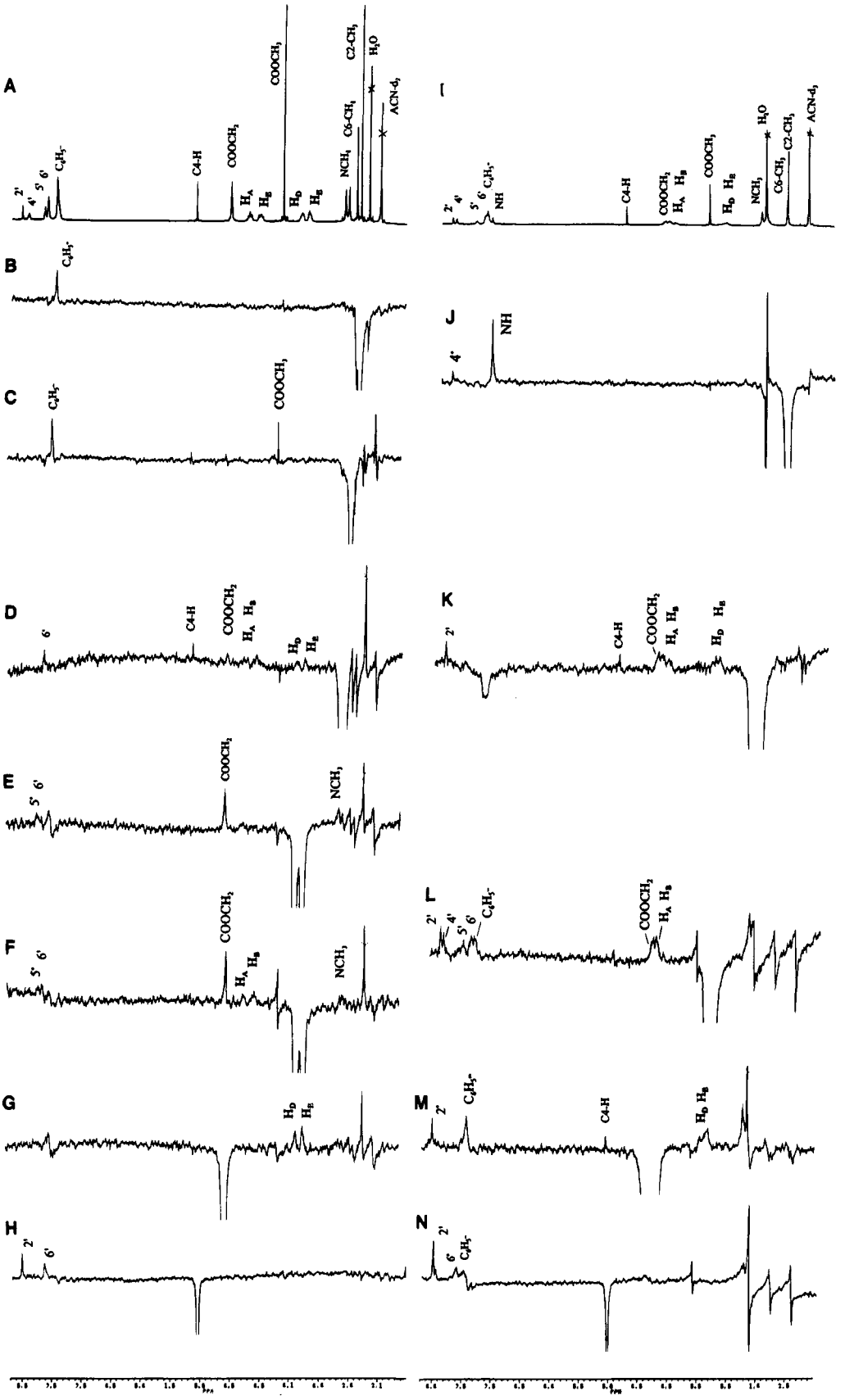


Figure 7. ^{1}H NMR spectra and representative NOE difference spectra of nicardipine and its Ca^{2+} complex in CD_3CN at 30 ± 1 °C: (A) ^{1}H NMR spectrum of the free drug; (B–H) NOE difference spectra in the free drug obtained by setting the decoupler frequency to irradiate C2-CH_3 (2.26 ppm), C6-CH_3 (2.33 ppm), NCH_3 (2.49 ppm), H_D (3.26 ppm), H_E (3.15 ppm), $COOCH_2$ (4.47 ppm), and C4-H (5.05 ppm); (I) ^{1}H NMR spectrum of nicardipine + 1.5 molar equiv of Ca^{2+}; (J–N) NOE difference spectra in the drug:Ca^{2+} complex caused, respectively, by irradiation of C2,6-CH_3 (2.29 ppm), NCH_3 (2.72 ppm), H_D/H_E (3.31 ppm), $COOCH_2$ (4.37 ppm), and C4-H (5.03 ppm). The residual water and CH_3CN protons are indicated by X.

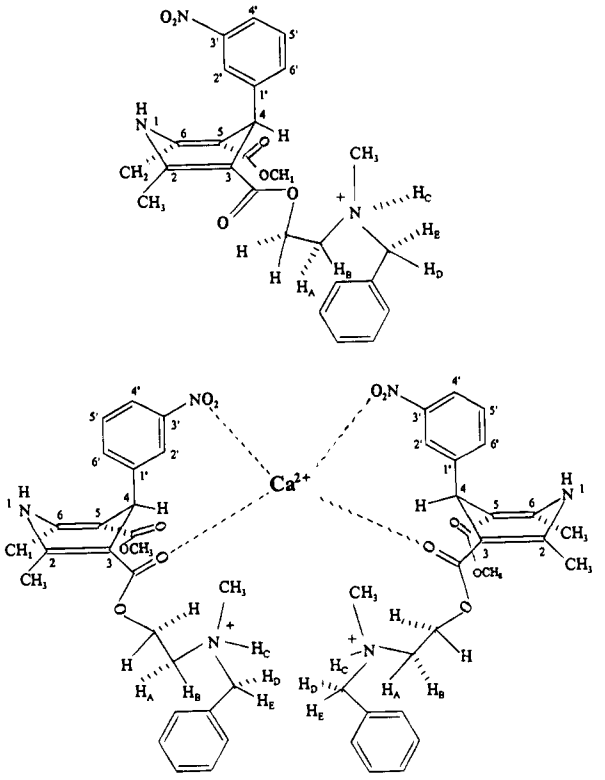


Figure 8. Molecular models of nicardipine (top) and its calcium "sandwich" complex (bottom) derived from 1D-NOE data.

and H-2′ protons are observed (Figure 7M). The close spacial proximity of the H-2′, C4-H, and $COOCH_2$ protons is thus suggested, indicating the formation of a sp-rotamer. The relatively much larger enhancement of the C_6H_5 protons compared to the free drug (Figure 7G,M) is likely due to the proximity of the phenyl ring of the second drug molecule in the Ca^{2+} sandwich complex (Figure 8).

Upon irradiation of C4-H proton, NOEs from H-2′ and H-6′ which are both proximal to C4-H protons appear in both the free drug and the Ca^{2+} complex (Figure 7H,N). In the Ca^{2+}-bound form, the increased H-2′ signal and, additionally, small NOE from C_6H_5 protons suggest that the rotational freedom around C4−C1′ bond is restricted due to a preference for the sp-rotamer.

Discussion

A great deal of research has been conducted toward ascertaining the active conformation of the DHPs as calcium entry blockers.[4,20] To this end, much effort has gone into synthesizing new DHPs with various substituents on the 4-phenyl ring and at the 2,3,5,6-positions of the dihydropyridine ring.[7b,21] It is now largely accepted that the active conformation of the DHPs involves a flat-boat shape for the 1,4-dihydropyridine ring with a pseudoaxial orientation of the 4-aryl ring[22] and that a correlation between the degree of the DHP ring flattening and pharmacological activity exists.[4b,22a] Most of the studies on DHPs have shown that, both the sp- and ap-rotamers exist with no preference for one over the other.[4b,21c] In contrast, our NMR results on nicardipine show that in CD_3CN solution the ap-rotamer with the C3 side chain folded toward the DHP ring is dominant. This folding is probably due to $\pi-\pi$ interactions between the C_6H_5 and C4-aryl moieties.

The binding of Ca^{2+} ions causes a significant conformational change in nicardipine as judged from the difference absorption and NMR spectral data. The resulting conformation appears to be relatively more rigid than the neat drug as judged from the larger line widths of many protons in the Ca^{2+}-bound drug (Figure 4B). Analysis of the binding isotherms from the difference spectroscopy (Figure 2) and 1D-NOE data (Figure 7) show a predominantly 2:1 drug:Ca^{2+} complex. The difference spectral data show that Ca^{2+} binding affects both the DHP and phenyl rings and suggest that these moieties may be brought close together in the cation complex. This is also evident from comparison of the 1D-NOE data, particularly upon irradiation of the NCH_3 protons in the free and Ca^{2+}-bound drug. While in the free nicardipine, a relatively small NOE from H-6′ proton in noticed, in the drug containing Ca^{2+} the disappearance of H-6′ and appearance of H-2′ NOE is observed. That the C4-aryl and the C3 side chain attached to the DHP ring are more proximal in the Ca^{2+} complex than in the free drug is also supported by the ^1H and ^{13}C NMR results, which demonstrate that H-2′ and NCH_3 protons are significantly affected by the presence of the metal ion (Figure 5A,C). We believe that the attraction is achieved *via* the chelation of the metal ion by the *m*-NO_2 and the C3-$COOCH_2$ carbonyl groups. The model shown in Figure 8 attempts to illustrate the features of the free and Ca^{2+}-bound "sandwich" complex of nicardipine using the information derived from the above spectral data.

Our attempts at making a similar detailed study on nifedipine were not very successful mainly since Ca^{2+} binding had a weaker effect on the chemical shifts of hydrogen and carbon atoms in this molecule. The $\Delta\delta$s did not exceed 0.04 ppm in either ^1H and ^{13}C resonances. However, a trend indicating saturation at about 0.5 Ca^{2+} mole ratio was observed in most of the curves plotted (data not shown). The most significant 1D-NOE results were obtained upon irradiation of H-1 proton (at 7.12 ppm) (Figure 9). Thus, in the free drug a small NOE from 4′ and 5′ protons appears, while in the presence of Ca^{2+} an additional positive NOE arising from H-3 proton is observed. This result suggest that in the presence of the metal ion there is a preference for the sp-rotamer as in the case of nicardipine. A main difference between the two DHPs is the presence of the $\pi-\pi$ interaction between the C_6H_5 and C4-aryl rings in nicardipine which contributes to a rigid conformation of the molecule in both the Ca^{2+}-bound and unbound forms.

In a recent study on the conformational and dynamic features of nimodipine [3-[(2-methoxyethoxy)carbonyl]-5-(isopropoxycarbonyl)-4-(3-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine] in [$^2H_6$]dimethyl sulfoxide, Gaggelli *et al.*[14a] demonstrated that the C3 and C5 side chains fold in opposite directions with respect to the DHP ring and that molecular motions affect atoms within the flexible chains at a relatively short distance from the DHP ring. Moreover, the authors have shown that the C3-(2-methoxyethoxy)carbonyl substituent folds back in a way that brings the CH_2 protons in the chain in proximity to C4-H. This orientation is, in fact, present in the case of nicardipine solution in the presence of Ca^{2+}, as indicated by the 1D-NOE data (Figure 7M).

In conclusion, we have established that, in solution,

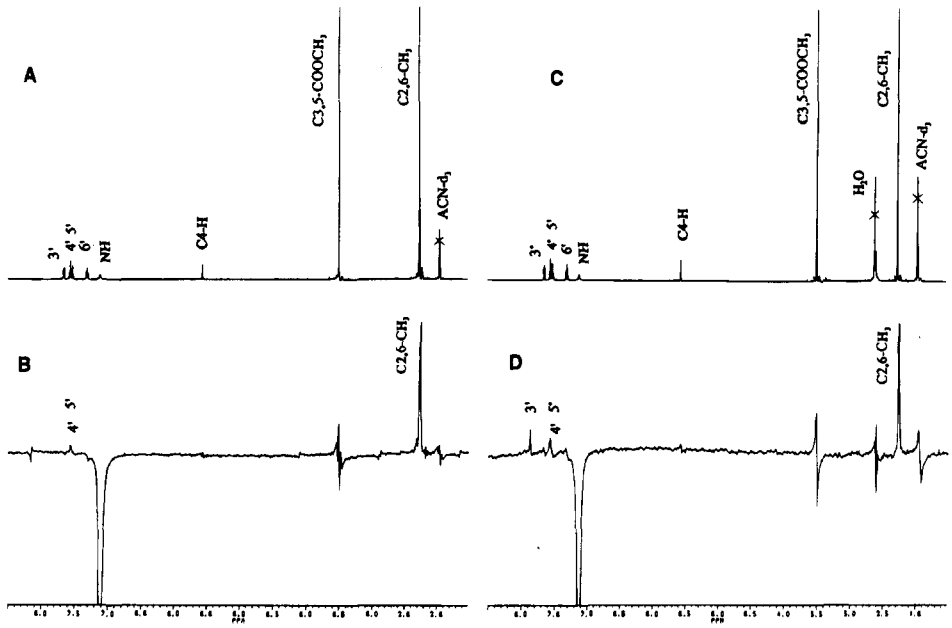


Figure 9. ^1H NMR spectra and representative NOE difference spectra of nifedipine and its Ca^{2+} complex in CD_3CN at 30 ± 1 °C: (A) ^1H NMR spectrum of the free drug; (B) NOE difference spectrum in the free drug obtained by irradiation of NH proton; (C) ^1H NMR spectrum of nifedipine + 1.0 molar equiv of Ca^{2+}; (D) NOE difference spectra in the drug:Ca^{2+} complex caused by irradiation of NH proton.

nicardipine exists mainly as a rigid ap-rotamer unlike other DHPs.[4b,18] In addition, we report the first example of a stable sp-rotamer in a DHP drug arising by coordination of Ca^{2+} to the donor groups in the drug molecule. As in the case of verapamil[10] and diltiazem,[11] nicardipine also forms a 2:1 drug:Ca^{2+} sandwich complex. In light of our findings,[11,12] we suggest that the Ca^{2+}-bound form of the DHP drugs may represent its biological active form in the nonpolar milieu of the lipid bilayer.[12] We also visualize a drug–Ca^{2+}–receptor ternary complex as an intermediate in the signal transduction process. Attempts to experimentally demonstrate such a complex are underway in our laboratory using the purified receptor.

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