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Calcium Antagonism and Structure-affinity Relationships of Terfenadine, a Histamine H_1 Antagonist, and Some Related Compounds

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Abstract—Calcium channel affinity of terfenadine and its optical isomers was determined by the displacement of [^{3}H]nitrendipine on rat cerebral cortex membranes. Terfenadine showed a pK_d of $6{\cdot}36 \pm 0{\cdot}03$ whereas its *R*(+)-isomer (VUF4567) had a pK_d value of $6{\cdot}39 \pm 0{\cdot}03$ and the *S*(−)-isomer (VUF4568) had a pK_d of $6{\cdot}40 \pm 0{\cdot}04$. The same affinity between the enantiomers suggests that the binding domain on the membrane is not sterically restricted towards the part of the molecule in which the chiral centre is present. The characteristics of terfenadine in regulating [^{3}H]nitrendipine binding were similar to those of some other diphenyl-alkylamine type calcium antagonists. It allosterically altered the binding affinity for nitrendipine and acted at the same site linked to the calcium channel as gallopamil. A structure-affinity relationship among a group of terfenadine analogues is discussed.

Calcium antagonists are an important class of biologically active compounds which inhibit a variety of biological processes regulated by calcium channels such as muscle contraction, hormone secretion and neurotransmitter release. Recently it has been demonstrated that some calcium antagonists are capable of relaxing bronchial smooth muscle (Joubert 1990). Thus, such compounds have potential value for the management of asthma. Structurally, calcium antagonists consist of heterogeneous organic compounds which can be classified into four major groups: 1,4-dihydropyridines, phenylalkylamines, benzothiazepines, and diphenylalkylamines (Godfraind et al 1986). Of the four groups, the diphenylalkylamines has received the least attention as regards structure-activity relationships.

Terfenadine, a non-sedating histamine H_1-receptor antagonist, has been shown to inhibit dose-dependently antigen-induced bronchoconstriction in actively sensitized guinea-pigs (Tasaka et al 1988). Structurally, terfenadine is similar to the diphenylalkylamines and a calcium channel antagonism by terfenadine could be responsible for this effect.

The present study was carried out to investigate the calcium channel affinity of terfenadine, its optical isomers and some related compounds (Fig. 1) by the displacement of [^{3}H]nitrendipine, to gain insights on the structure-affinity relationships and stereoselectivity of the diphenylalkylamine-type calcium antagonists. We also evaluated the interactions of gallopamil, a phenylalkylamine-type calcium antagonist, with terfenadine in regulating [^{3}H]nitrendipine binding in order to determine whether these two compounds act at the same site.

Materials and Methods

Drugs

[^{3}H]Nitrendipine (73 Ci mmol^{-1}) with a radiochemical purity of more than 98·5% was purchased from New England Nuclear, USA. Gallopamil was obtained from Knoll AG, Germany and nifedipine from Bayer AG, Germany. Terfenadine was synthesized according to the method of Carr & Meyer (1982). Terfenadine enantiomers (VUF4567, VUF4568) were synthesized as previously described (Zhang et al 1991a) and were chromatographically pure. The enantiomer excess of both isomers, determined by ^{31}P NMR spectroscopy of the derivatized compound with Anderson-Shapiro reagent (Anderson & Shapiro 1984), was close to 100%. Terfenadine was a racemic mixture of *R*-isomer/*S*-isomer = 48/52.

The synthesis of VUF4585 and VUF4589 has previously been described (Zhang et al 1991b). Both compounds were chromatographically pure. Their physicochemical properties are listed below. Melting points were determined on a Mettler FP5 melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker WH-90 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and coupling constants are in Hz. Mass spectral data were registered on a Finnigan MAT 90 mass spectrometer with electron impact (EI) ionization. VUF4591 was obtained by the synthesis illustrated in Fig. 2 and was chromatographically pure. Dynagel liquid scintillation fluid was procured from Baker Netherlands. All other chemicals were of analytical grade purity.

1-{4-[4-(1,1-Dimethylethyl)phenyl]-4-hydroxybutyl}-α-phenyl-4-piperidinomethanol (VUF4585)

93·7–94·3°C (acetone); ^{1}H NMR ($CDCl_3$) δ (ppm): 1·31 (s, 9H, CH_3), 1·65 (m, 4H, piperidine $C_{3',5'}$-H), 1·76–2·17 (m, 6H, piperidine $C_{2',6'}$-H_{ax} and -CH_2CH_2CH(OH)Ph), 2·39 (t, 2H, J = 6·7 Hz, NCH_2), 2·85 and 3·18 (m, 2H, piperidine $C_{2',6'}$-H_{eq}), 3·02 (m, 1H, piperidine $C_{4'}$-H), 4·34 (d, 1H, J = 7·2 Hz, PhCH(OH)-piperidine), 4·60 (m, 1H, -CH_2CH_2CH(OH)Ph), 7·30 (m, 9H, aromatic H); MS *m/e*: 395.

α,α-Diphenyl-1-methyl-4-piperidinomethanol (VUF4589)

Melting point 134·0–134·7°C (acetone); ^{1}H NMR ($CDCl_3$) δ (ppm): 1·49–1·70 (m, 4H, piperidine $C_{3',5'}$-H), 1·94 (m, 2H,

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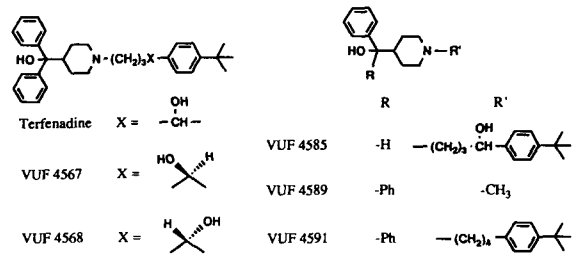

FIG. 1. The structures of terfenadine, its optical isomers and some related compounds.

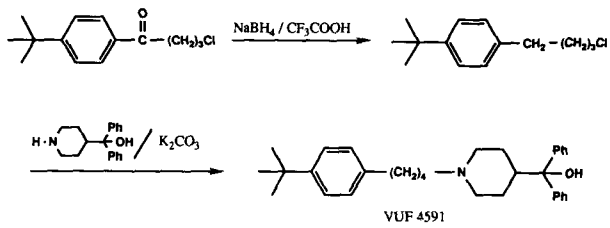

FIG. 2. Synthesis of VUF4591.

piperidine $C_{2',6'}$-H_{ax}), 2·24 (s, 3H, CH_3), 2·41 (m, 1H, piperidine CH_4-H), 2·88 (m, 2H, piperidine $C_{2',6'}$-H_{eq}), 7·13–7·51 (m, 10H, aromatic H); MS *m/e*: 281.

1-{4-[4-(1,1-Dimethylethyl)-phenyl]butyl}-α,α-diphenyl-4-piperidinomethanol (VUF4591)

To 50 mL trifluoroacetic acid stirred at 0°C under nitrogen was added 4·56 g (0·12 mol) $NaBH_4$ over 30 min. To this mixture at 15°C was added dropwise over 30 min a solution of 4·77 g (0·02 mol) 4-chloro-1-[4-(1,1-dimethylethyl)phenyl]-1-butanone in 50 mL dichloromethane. After stirring at room temperature (21°C) overnight, the mixture was diluted by adding 100 mL H_2O and neutralized with 10% NaOH at 0°C. The dichloromethane layer was separated and the water layer was extracted with petroleum ether (40–60°C) twice. The combined organic solution was dried with Na_2SO_4 and evaporated to dryness. The remaining colourless oil containing 4-[4-(1,1-dimethylethyl)phenyl]-1-chlorobutane was used without further purification. ^{1}H NMR ($CDCl_3$) δ (ppm): 1·31 (s, 9H, CH_3), 1·80 (m, 4H, $-CH_2-CH_2-$), 2·62 (t, 2H, Ph-CH_2-, J = 7·0 Hz), 3·55 (t, 2H, $ClCH_2$-, J = 6·5 Hz), 7·10–7·35 (m, 4H, aromatic H).

The 1-chlorobutane obtained above was dissolved in 250 mL dry acetone. To the solution was added 6·0 g (0·04 mol) NaI (dried at 150°C overnight). The solution was then refluxed for 6 h. After evaporation to dryness, the residue was extracted with petroleum ether (40–60°C) (100 mL × 3). The combined petroleum ether layer was dried with Na_2SO_4 and evaporated to dryness. The residue was then dissolved in 300 mL butanone-2. To this solution was added 5·32 g (0·02 mol) α,α-diphenyl-4-piperidinomethanol and 2·76 g (0·02 mol) K_2CO_3. The mixture was refluxed with stirring overnight. After evaporating to dryness, the residue was taken up with dichloromethane. The dichloromethane solution was dried with Na_2SO_4 and evaporated to dryness yielding a slightly brown oil. Purification by a silica gel column (ethyl acetate/petroleum ether (40–60°C) 2:1 saturated with NH_3) furnished a thick colourless oil which was subsequently crystallized from acetone. Yield: 44%. Melting point 126·2–127·0°C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1·29 (s, 9H, CH_3), 1·47–1·64 (m, 8H, piperidine $C_{3',5'}$-H and -$(CH_2)_2$-), 1·94 (m, 2H, piperidine $C_{2',6'}$-H_{ax}), 2·33 (t, 2H, J = 7 H, NCH_2), 2·47 (m, 1H, piperidine C_4-H), 2·59 (t, 2H, J = 7 Hz, CH_2Ph), 2·97 (m, 2H, piperidine $C_{2',6'}$-H_{eq}), 7·09–7·51 (m, 14H, aromatic H); MS *m/e*: 455.

For the binding studies, all compounds were used as free base and were dissolved in 99% (v/v) dimethylsulphoxide (DMSO) and diluted with 50 mM Tris-HCl (pH 7·4) buffer to the desired concentrations. The final concentrations of DMSO in the incubation solution were 1% (v/v) at maximum.

Membrane preparation

The preparation of rat cerebral cortex membrane was based on that described by Bast et al (1987). The cerebral cortices from male Wistar rats, 220–240 g, were isolated and homogenized in ice-cold Tris-HCl buffer (50 mM, pH 7·4) in a ratio of 1:3 (w/w). The homogenate was centrifuged (48 000 *g* for 10 min) and centrifugation was repeated 3 times with intermittent resuspension of the pellet in the buffer and stored at −80°C until further use.

[^{3}H]Nitrendipine binding assay

The membrane suspension of the rat cerebral cortex (180 μg protein mL^{-1}) was incubated with the indicated compounds for 60 min at 37°C in 50 mM Tris-HCl buffer (pH 7·4 at 37°C). Incubation volume was 0·5 mL and [^{3}H]nitrendipine concentration was 0·25 nM. The reaction was stopped by the addition of 4 mL ice-cold Tris-HCl buffer (pH 7·4 at 0°C), followed by immediate filtration under reduced pressure onto Whatman GF/C filters. The filters were washed twice with 4 mL cold buffer. The retained radioactivity was counted with a Packard liquid scintillation counter after addition of 5 mL scintillation fluid to the filters. Each experiment was performed in triplicate. In the saturation experiment, 1 μM nifedipine was used for non-specific binding. Because of photolability of dihydropyridines, binding experiments were performed under a sodium lamp.

Interaction between gallopamil and terfenadine

Rat cerebral cortex membrane (180 μg protein mL^{-1}) was incubated with 0·25 nM [^{3}H]nitrendipine and increasing concentrations of gallopamil in the absence or presence of 1·0, 1·75, 2·5 or 10·0 μM of terfenadine. Incubation time was 60 min at 37°C and incubation volume was 0·5 mL. Other conditions were the same as described above.

Data analysis

The binding data were evaluated using the nonlinear iterative curve-fitting program LIGAND (Munson & Rodbard 1980). This program is based on the law of mass action for multiple binding sites and provided a correction for free ligand concentrations and non-specific binding. The dissociation constants were obtained by simultaneous curve fitting of a number of independent experiments.

Results

Fig. 3 shows the displacement curves of specifically bound

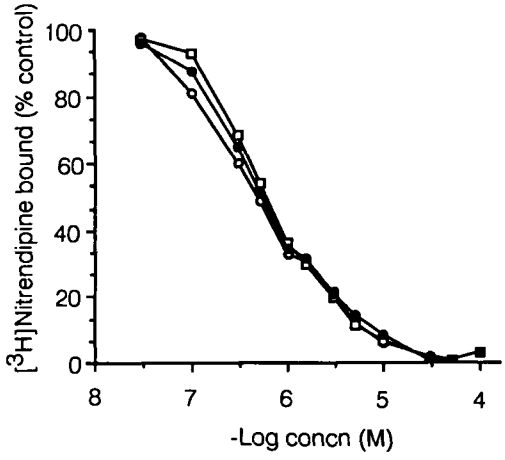

FIG. 3. Displacement curves of specifically bound [^{3}H]nitrendipine from rat cerebral cortex membranes by terfenadine and its optical isomers. [^{3}H]Nitrendipine concentration was 0·25 nM. Non-specific binding was determined with 1000 nM nifedipine. The experiment was replicated three times with similar results. ○, VUF4567; □, VUF4568; ●, terfenadine.

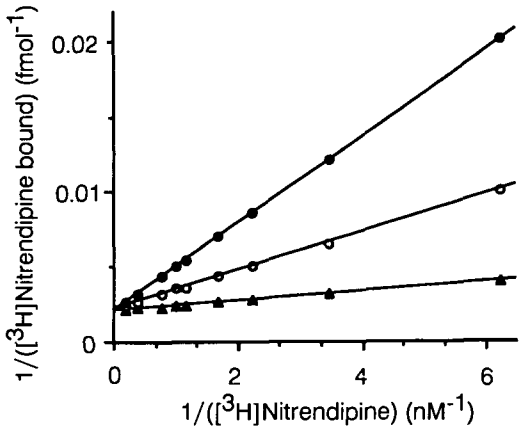

FIG. 4. Double-reciprocal plot of [^{3}H]nitrendipine binding in the absence (▲) or presence of 4×10^{-7} M (○) or 2×10^{6} M (●) terfenadine. Data are the means of triplicate determinations from a typical experiment that was replicated three times with similar results. The K_d for each condition is as follows: ▲, 0·019; ○, 0·549; ●, 0·710 nM.

[^{3}H]nitrendipine from rat cerebral cortex membranes by terfenadine and its optical isomers VUF4567 and VUF4568. Unlike phenylalkylamine-type compounds, terfenadine and its optical isomers had higher affinity for the nitrendipine binding site than, for example, gallopamil (see Fig. 5) and caused almost 100% inhibition of nitrendipine binding. The negative logarithms of dissociation constants (pK_d) for terfenadine, VUF4567 and VUF4568 were $6{\cdot}36 \pm 0{\cdot}03$, $6{\cdot}39 \pm 0{\cdot}03$ and $6{\cdot}40 \pm 0{\cdot}04$, respectively. There is apparently no difference in affinity with the calcium channel between the optical isomers of terfenadine. In fact, when the chiral alcohol function was replaced by a methylene group, the affinity of the resulting compound VUF4591 was increased more than 6-fold (Table 1). Changing the *N*-substituent on the piperidine ring to a methyl group reduced affinity by 60 times (VUF4589), whilst replacing one of the phenyl rings at the diphenylmethanol part with a hydrogen atom reduced the affinity by 20 times (VUF4585). These data suggest that for this group of compounds all three phenyl rings are important for the affinity of the compounds to the receptor.

A double-reciprocal plot of [^{3}H]nitrendipine binding to membranes in the presence of different concentrations of terfenadine (Fig. 4) indicated that terfenadine reduced the K_d of nitrendipine for its site on the calcium channel. The maximal effect of terfenadine was to reduce the K_d for [^{3}H]nitrendipine approximately 37 times. Greater quantities of terfenadine did not reduce the apparent K_d below this limit. Although this finding may suggest a competitive inhibition of [^{3}H]nitrendipine binding by terfenadine, it does not reveal whether this competition occurs at the same site or an allosteric site. Thus we carried out the next experiment to investigate the influence of gallopamil on terfenadine in regulating [^{3}H]nitrendipine binding.

As shown in Fig. 5, gallopamil was able to reverse the inhibition of [^{3}H]nitrendipine binding caused by terfenadine. At the concentration of terfenadine which caused about 50% inhibition of [^{3}H]nitrendipine binding, gallopamil was able

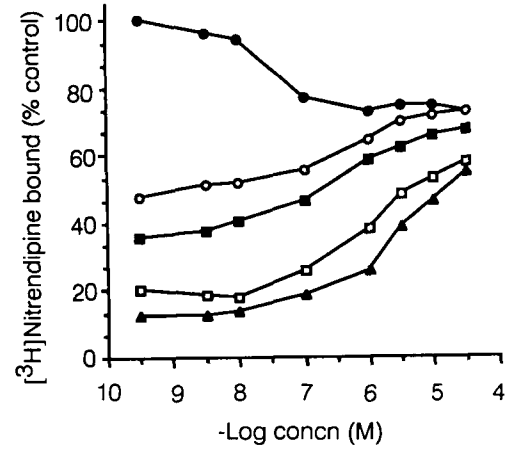

FIG. 5. Gallopamil reversal of the inhibition of [^{3}H]nitrendipine binding produced by terfenadine. Concentration-response curves for the influence of gallopamil on [^{3}H]nitrendipine binding to membranes were obtained with eight concentrations of gallopamil and 0·25 nM [^{3}H]nitrendipine. [^{3}H]Nitrendipine binding was determined at each concentration of gallopamil in the absence (●) or the presence of terfenadine at 1·0 (○), 1·75 (■), 2·5 (□) and 10·0 (▲) μM. Values are presented as the percentage of control [^{3}H]nitrendipine binding determined in the absence of all inhibiting agents. The experiment was replicated twice with similar results.

Table 1. Effects of terfenadine and its related compounds on [^{3}H]nitrendipine binding in rat cerebral cortex membanes.

Compounds	pK_d (mean ± s.d.)
Terfenadine	$6{\cdot}36 \pm 0{\cdot}03$
VUF4567	$6{\cdot}39 \pm 0{\cdot}03$
VUF4568	$6{\cdot}40 \pm 0{\cdot}04$
VUF4585	$5{\cdot}06 \pm 0{\cdot}02$
VUF4589	$4{\cdot}57 \pm 0{\cdot}03$
VUF4591	$7{\cdot}18 \pm 0{\cdot}01$

The data are the means ± s.d. of three independent experiments.

to bring the level of [^{3}H]nitrendipine binding back to that of the control. When higher concentrations of terfenadine were present, the EC50 for gallopamil-induced restoration of [^{3}H]nitrendipine binding was shifted to the right. For example, at a terfenadine concentration of 1·0 μM, the EC50 for gallopamil-induced restoration of [^{3}H]nitrendipine binding was approximately 3×10^{-7} M whilst at a terfenadine concentration of 2·5 μM, the EC50 for gallopamil to restore [^{3}H]nitrendipine binding to the maximal 80% of the control was 10^{-6} M. These data demonstrated a competition between gallopamil and terfenadine in regulating [^{3}H]nitrendipine binding. Since gallopamil has been well documented (Murphy et al 1984) to influence [^{3}H]nitrendipine binding allosterically, the inhibition of [^{3}H]nitrendipine binding caused by terfenadine seems to be of the same type.

Discussion

It is known that all other three classes of calcium antagonists—dihydropyridines, phenylalkylamines and benzothiazepines—show pronounced stereoselective activity (Triggle & Janis 1987). For example, in various binding systems the active enantiomer of nitrendipine showed more than 15- to 20-fold higher affinity than the inactive enantiomer, whilst the active enantiomer of gallopamil exhibited 3- to 26-fold higher affinity than the inactive one. Diltiazem is in fact an optically pure compound. However, so far there have been no reports on the stereoselectivity of diphenylalkylamine-type calcium antagonists. In this study both terfenadine enantiomers showed the same potency in the inhibition of [^{3}H]nitrendipine binding. This suggests that the binding domain linked to the calcium channel on the membrane is not sterically restricted towards this part of the molecule. When the chiral centre was abrogated, the K_d value of the corresponding compound VUF4591 was less than one-sixth that of terfenadine (Table 1).

Structure-affinity relationship studies revealed that all three phenyl rings in this type of compound are important for high affinity to the calcium channel. Removing any one of them caused a dramatic decrease in affinity (Table 1).

The present study demonstrated that terfenadine is a dual antagonist on histamine H_1 receptors and voltage-dependent calcium channels. The characteristics of terfenadine in regulating [^{3}H]nitrendipine binding were consistent with those of other diphenylalkylamine-type calcium antagonists, e.g. flunarizine and cinnarizine (Murphy et al 1984). Since the inhibition of [^{3}H]nitrendipine binding caused by terfenadine can be reversed by gallopamil (Fig. 5), the influence of terfenadine on [^{3}H]nitrendipine binding is apparently through an allosteric site. However, although terfenadine appeared to bind to the same site of the calcium channel as gallopamil, this type of calcium antagonist is different from phenylalkylamines since they were able to displace [^{3}H]-nitrendipine binding by almost 100%. This is also supported by the necessity of the third phenyl ring for the high affinity of this type of antagonist (see Table 1, terfenadine vs VUF4585).

The calcium antagonism by terfenadine probably contributes to the moderate effect of this drug in mild asthma. It might also be true that its calcium antagonism is responsible for some side-effects of this drug, e.g. headache and the cardiovascular effects observed when terfenadine is overdosed (McTavish et al 1990).

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