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Comparison of Crystallographic and Quantum Mechanical Analysis with Biological Data on Clonidine and Some Related Analogues

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SUMMARY

X-ray structure determinations and complete neglect of differential overlap, self-consistent field (CNDO/2) and perturbative configuration interaction using localized orbitals (PCILO) calculations of clonidine-related *alpha*-adrenergic molecules are presented and compared with the variation in blood pressure and heart rate induced by these molecules after intravenous injection in normotensive and pithed rats. Affinities on washed rat brain membranes determined by competition with the *alpha*$_2$-ligand [^{3}H] *p*-aminoclonidine are also given. X-ray results and quantum mechanical calculations confirm previous NMR data and indicate that for xylazine, tiamenidine, and 2-(2,6-dichlorophenyl)-1-methyl guanidine (IPRO-4) the C═N bond is exocyclic and consequently that the imino form predominates in the neutral molecules. For neutral lofexidine the C═N bond can only be endocyclic, and no prototropy occurs. For protonated clonidine and IPRO-4, about one-half of the positive charge is located on C(7) and the remaining charge is evenly distributed on the three H atoms of the guanidine function. In the xylazine molecule, most of the positive charge (0.37) is located on the endocyclic H-N(2). In the protonated lofexidine, the majority of the positive charge (0.27) is delocalized on C(7) but less (about 0.20) on H-N(2) and H-N(3). Apart from lofexidine, all of the molecules are characterized by a torsion angle ψ between the guanidine and the aromatic parts. For clonidine, the value of this angle ($\psi = 74°$) as calculated by PCILO agrees perfectly with X-ray results. Similar data were obtained from xylazine, tiamenidine, and IPRO-4. For lofexidine, the existence of three torsion angles makes the situation more complex, although very similar values were found between PCILO calculations and X-ray determinations. Biological data observed tallied well the literature. A good correlation ($r = 0.99$) was found between the hypotensive and the bradycardic activity, both of which were obtained by means of i.v. injections into anesthetized, normotensive rats. K_i values obtained on rat brain membranes were strongly linked with the hypertensive activity and much less satisfactorily with the hypotensive activity, supporting the view that *alpha*$_2$-receptors are involved in hypertensive activity. Finally, X-ray analysis and PCILO calculations clearly demonstrate the striking resemblance between the five molecules examined and strongly support our hypothetical model for the interaction of clonidine-like imidazolidines and the *alpha*-adrenoceptor.

INTRODUCTION

In previous work, we proposed a structure for clonidine (1) (Figs. 1 and 9) and showed that it had the requisite characteristic features to interact with an *alpha*-receptor model (2). Additional ^{1}H- and ^{13}C-NMR studies (3–5) have confirmed our spectral assignments.

Alpha-receptor models have been proposed by Belleau (6), Kier (7), and Pullman *et al.* (8). Recently (9) the *alpha*-adrenergic mechanism for the hypotensive activity of the phenylimidazolidines molecules and the similarity between central and peripheral *alpha*-adrenergic receptors have been established. Since then, numerous clonidine-like derivatives have appeared, notably xylazine (10), lofexidine (11), tiamenidine (12), and IPRO-4[4] (13), a molecule that we have synthesized (Fig. 1). The purpose

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[4] The abbreviations used are: IPRO-4, 2-(2,6-dichlorophenyl)-1-methyl guanidine; CNDO/2, complete neglect of differential overlap, self-consistent field; PCILO, perturbative configuration interaction using localized orbitals; [^{3}H]PAC, ^{3}H-labeled *p*-aminoclonidine.

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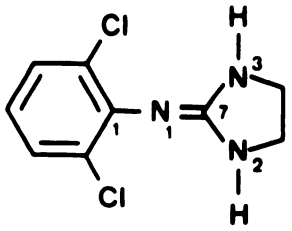
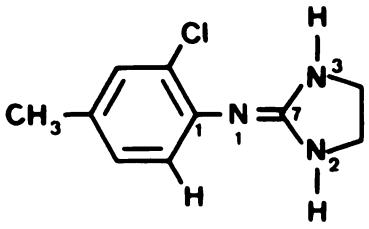
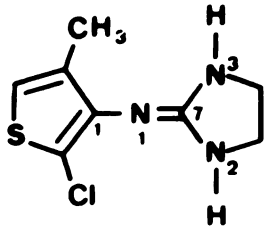


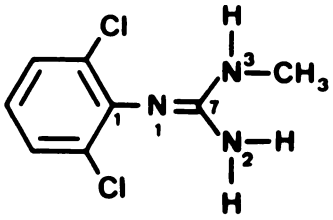
Clonidine



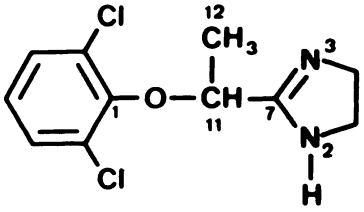
Tolonidine



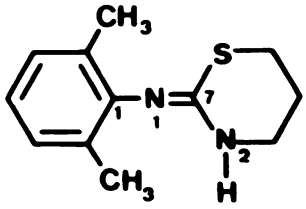
Tiamenidine



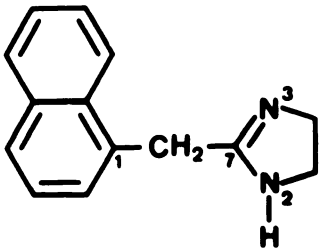
IPRO – 4



Lofexidine



Xylazine



Naphazoline

FIG. 1. *Clonidine and some related analogues*
Only atoms discussed in the text have been numbered.

of this work was to determine whether the similarity in the pharmacological profile corresponds to common structural features.

In this respect, X-ray diffraction results, CNDO/2 and PCILO calculations for the molecules are presented together with their biological data, i.e., the variation in blood pressure and heart rate following intravenous injection into normotensive and pithed rats and affinities on washed rat brain membranes determined by competition with the *alpha*$_2$-ligand-*p*-aminoclonidine [^{3}H]PAC.

METHODS

Crystal Structure Analysis

Intensity data were collected on a fully automated-Enraf-Nonius CAD-4 diffractometer using graphite monochromated CuKα radiation. All structures were solved either by direct methods or by Patterson techniques and refined with a diagonal-matrix least-squares procedure. Detailed crystallographic data and descriptions of the conformations and molecular interactions of the drugs

presented here have been published in specialized journals. No comparison of these structures has hitherto been attempted.

CNDO/2 Calculations

In order to determine bond orders and net charges on the atoms of the guanidine functions, CNDO/2 calculations were performed for the free organic cations according to the method of Pople and Segal (14). The crystallographic coordinates of atoms were used as input data. In addition, theoretical dipole moments were calculated using the method of Pople and Segal (15).

PCILO Calculations

The crystal structures of the compounds were used as the geometrical input data for calculating conformational energy maps obtained by the PCILO method (16). Similar calculations have been performed by Pullman *et al.* (8, 17) on some phenethylamines and related compounds with *alpha*-adrenergic activity.

Animal Experiments: Blood Pressure and Heart Rate in Normotensive Rats and in Normotensive Pithed Rats

Male, normotensive Wistar rats (weight 250–300 g) were anesthetized with sodium pentobarbitone (50 mg/kg). Mean blood pressure and heart rate were measured with a transducer (Statham P23 DB) connected to the carotid artery and recorded on a Gilson M5 P polygraph. A jugular vein was cannulated for i.v. injections.

Hypotensive and bradycardic activities in normotensive rats. The hypotensive activity of the compounds was investigated after i.v. injections. A single dose of 0.1 ml/100 g of body weight was given to one animal only. The mean value (± standard deviation) of mean arterial pressure prior to any drug treatment was 114 ± 1.8 mm Hg (n = 92), and 351 ± 4.6 beats/min for heart frequency.

Hypertensive activities in pithed normotensive rats. The rats were pithed according to the method of Shipley and Tilden (18). The trachea was cannulated to allow artificial respiration. Drugs were injected at a volume of 0.05 ml/100 g of body weight, at intervals of not less than 5 min or when the blood pressure had reverted to its predose value. The mean value (± standard deviation) of mean arterial pressure prior to any drug treatment was 55.9 ± 1.5 mm Hg (n = 24). Pressor activity *in vivo* is expressed in pD'2, since pD2 as defined by Ariens and Van Rossum (19) applies only to the equilibrium possible in *in vitro* experiments.

Binding Assays

Binding assays were performed on rat cerebral cortex homogenate as previously described (20). Briefly stated, drug inhibition (IC_{50}) of [^{3}H]PAC binding (0.15 nM) using five or six concentrations of each unlabeled drug was determined graphically by log-probit analysis. Apparent K_i values were calculated from the equation

$$Ki = IC_{50}/1 + ([^3H]PAC/K_D),$$

where the mean K_D value was 0.69 ± 0.02 nM. K_i values reported in Table 3 are the mean of three independent experiments, each performed in triplicate.

Octanol Buffer—Partition Coefficients and pK_a Values

The apparent partition coefficient of the compounds between n-octanol and aqueous 0.1 M phosphate buffer (pH 7.4) was determined as a measure of over-all lipophilic behavior. Our method has already been described (21). pK_a values were determined in water using a Mettler memotitrator. The apparent partition coefficient and pK_a values of tiamenidine were kindly provided by Dr. Sistovaris, of the Hoechst Laboratory.

RESULTS

Crystal data. Some interesting interatomic distances related to the free bases and the protonated forms of the studied molecules are given in Table 1. Apart from xylazine, the C(1)—N(1) bond (refer to numbering scheme in Fig. 1) in the neutral forms is appreciably shorter than expected for a C-sp^2—N bond (1.426 Å) and tallies with a conjugation with the aromatic nucleus or the thienyl ring. N(1)—C(7) bonds (mean value 1.293 Å) are always shorter than the other two C(7)—N bonds, indicating that the C=N bond is exocyclic, and accordingly that the imino form of the neutral molecules predominates. This result fits with our earlier proposal for clonidine. All of the C(1)—N(1)—C(7) angles are <120°. C(7)-centered internal bond angles are lower than the external ones. When the protonated forms of the molecules are examined, the C(1)—N(1) bonds (mean value 1.425 Å) are in perfect accordance with the expected 1.426 Å value. In all molecules but lofexidine, the protonation occurs on the N(1) nitrogen atom. C—N bonds (mean value 1.325 Å) have similar lengths and range between single and double bonds. This indicates that the electrons are delocalized and that the positive charge is spread over the three nitrogen atoms of the guanidine function. The fact that the C(7)—N(2) bond is always shorter than C(7)—N(1) and C(7)—N(3) bonds is so far unaccounted for. In contrast with the neutral forms, the C(1)—N(1)—C(7) angles have values larger than 120° but, apart from IPRO-4 hydrochloride (N.B. IPRO-4 has an opened

TABLE 1

Comparison of some interatomic distances

The numbering of atoms is given in Fig. 1.

Molecule and form	Ref.	Interatomic distance[a] (Å)			
		C(1)—N(1)	N(1)—C(7)	C(7)—N(2)	C(7)—N(3)
Clonidine[b]					
Hydrochloride	22	1.418 (2)	1.328 (2)	1.322 (2)	1.321 (2)
Phosphate	23	1.420 (4)	1.337 (4)	1.318 (5)	1.333 (5)
Xylazine					
Base A[c]	24	1.421 (6)	1.283 (6)	1.360 (6)	—
Base B[c]	24	1.425 (8)	1.295 (6)	1.349 (6)	—
Phosphate	25	1.434 (3)	1.322 (3)	1.323 (3)	—
Tiamenidine					
Base	26	1.401 (9)	1.286 (9)	1.37 (1)	1.35 (1)
Hydrochloride	27	1.44 (1)	1.33 (1)	1.32 (1)	1.33 (1)
IPRO-4					
Base	28	1.389 (4)	1.308 (4)	1.339 (4)	1.364 (5)
Hydrochloride	28	1.412 (5)	1.349 (6)	1.312 (6)	1.338 (6)
Lofexidine					
Base	29	—	—	1.284 (8)	1.369 (8)
Hydrochloride	30	—	—	1.304 (7)	1.316 (7)

[a] Values in parentheses beside interatomic distances represent standard deviations.

[b] Attempts to solve the crystal structure of free-base clonidine were unsuccessful; we always obtained twin crystals.

[c] There are two independent molecules, A and B, in the cell.

guanidine function), the C(7)-centered internal valency angles are, as in the corresponding bases, smaller than the external ones.

In neutral lofexidine, where the C=N bond can only be endocyclic, the difference between C(7)—N(2) and C(7)—N(3) distances indicates that in the crystal the double bond is not delocalized on the two carbon-nitrogen bonds, and that no prototropy occurs. The same result has already been reported from NMR (^1H and ^{13}C) studies (5).

CNDO/2 calculations. Results of charge calculations carried out on the protonated molecules by the CNDO/2 method are given in Table 2. In the case of clonidine, about one-half of the positive charge is located on C(7) and the remaining charge is evenly distributed on the three hydrogen atoms of the guanidine function. The same result is observed for IPRO-4, in which N(2) bears two hydrogen atoms. In the xylazine molecule, most of the positive charge is located on the endocyclic H-N(2): 0.37, followed by 0.26 on C(7), 0.10 on C(8), and 0.17 on the exocyclic H-N(1). In protonated lofexidine, the majority of the positive charge (0.27) is delocalized on C(7), but less (about 0.20) on H-N(2), H-N(3), and C(1), respectively.

PCILO calculations. Apart from lofexidine, all of the molecules can be characterized by a dihedral angle ψ formed by the following two planes: Plane I, defined by C(2), C(1), and N(1); Plane II, defined by C(1), N(1), and C(7). This torsion angle *a priori* can adopt all discrete values between 0° (planar conformation) and 90° (perpendicular conformation. ψ is considered positive when, looking along the C(1)—N(1) bond, the far bond N(1)—C(7) rotates clockwise with respect to the near bond C(2)—C(1) (Fig. 2).

The conformational energy curve obtained for clonidine (Fig. 3) has been constructed as a function of the

TABLE 2

Electron charge density of the protonated molecules (CNDO/2 calculations)

In the case of free-base tiamenidine (26), electron density curves have shown the rotatability of the thienyl ring around the C(1)—N(1) bond; this can occur because the volume of a methyl group is nearly the same as that of a chlorine atom. The evidence implied a statistically significant occupancy of the substituted thienyl ring. Even if it was not clear from the Fourier maps in the case of the hydrochloride, it seems to us that the same phenomenon may occur. Consequently, the hydrogen atoms of the methyl group could not be located in either case. Since atomic coordinates are the input data for CNDO calculations, tiamenidine could not be dealt with.

Molecules[a]	$^{\delta}$C(7)	$^{\delta}$H-N(1)	$^{\delta}$H-N(2)	$^{\delta}$H-N(3)	Dipole moments (Debyes)
Clonidine					
Hydrochloride	0.45	0.17	0.17	0.17	—
Phosphate	0.46	0.18	0.18	0.18	47.2
Xylazine phosphate	0.26	0.17	0.37	—	—
IPRO-4 hydrochloride	0.46	0.18	0.19 × 2	0.17	57.4
Lofexidine hydrochloride	0.27	—	0.20	0.19	43.5

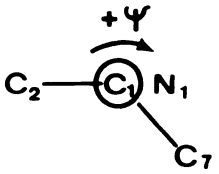


FIG. 2. *General definition of a torsion angle*

torsion angle ψ, for which an increment of 20° was used [ψ = 0 corresponds to the value obtained from crystal structure (i.e., 86° for the phosphate)]. This curve shows two energy minima. The first ($\Delta\psi \sim 20°$; i.e., ψ = 106° or 74°) corresponds to the value found in the hydrochloride crystal by Byre *et al.* (22). The second ($\Delta\psi$ = 200°; i.e., ψ = 286° = −74°) is symmetrical with the first and corresponds to a higher energy of 0.5 kcal/mole. Within the accuracy of the method, this result confirms that the solid-state conformation is the same as the free-ion conformation. PCILO conformational energy curves for xylazine, tiamenidine, and IPRO-4, very similar to that of clonidine, are not shown.

In the case of lofexidine, three angles can be varied (Fig. 4); but since a variation of three degrees of freedom in the PCILO method imply too extensive calculations and difficulties in locating the local energy minima, only two torsion angles were varied at a time. In the first energy curve, α and β were varied simultaneously while γ was fixed at its crystal value (γ crystal = 169°) (Fig. 4).

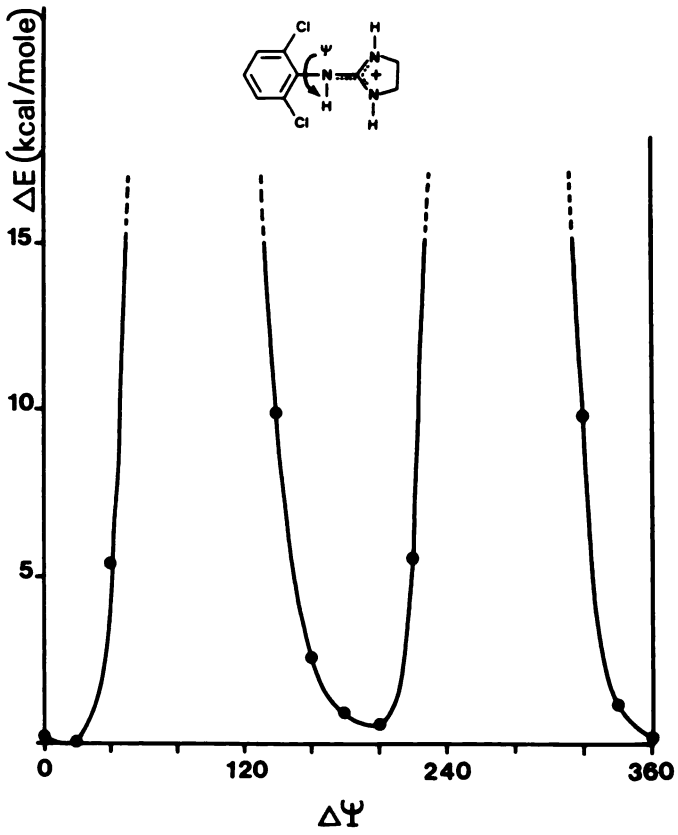


FIG. 3. *PCILO conformational energy curve of clonidine with the geometrical input data form*

Iso-energy curve in kilocalories per mole with respect to the global energy minimum taken as energy zero.

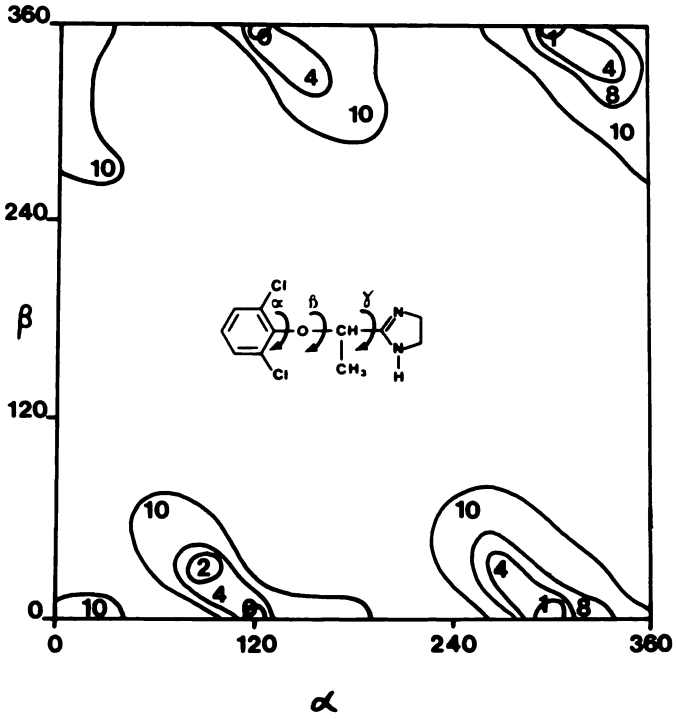


FIG. 4. *First PCILO conformational energy map of lofexidine with initial values α crystal = 68° and β crystal = 235°; γ crystal = 169° = constant*

Iso-energy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero.

Two energy minima were obtained, the first for $\alpha = \alpha$ crystal + 120°, $\beta = \beta$ crystal, and $\Delta E = 0$, and the second for $\alpha = \alpha$ crystal + 300°, $\beta = \beta$ crystal, and $\Delta E = 1.4$ kcal/mole. These two equivalent minima correspond to $\alpha \sim 0°$ or 180° and $\beta = 235°$. In the second case, β and γ were varied while α was maintained constant at α crystal = 68° (Fig. 5). Here again, two energy minima were obtained, the first for $\beta = \beta$ crystal, $\gamma = \gamma$ crystal, and $\Delta E = 0$, and the second for $\beta = \beta$ crystal − 30°, $\gamma = \gamma$ crystal + 180°, and $\Delta E = 0$. Within the limits of accuracy, these two equivalent minima correspond to $\beta \sim 235°$ and $\gamma \sim 0°$ or 180°. Results from Fig. 4 and Fig. 5 show without ambiguity that β remains the same in both curves and has the same value as that found in the crystal, i.e., $\beta \sim 235°$. Consequently, we were able to correlate the variations of α and γ obtained in the first and second analyses, respectively. In conclusion, it appears that α can vary between 60° and 180° (68° in the crystal) while β remains constant at 235° and γ can adopt the two symmetrical values $\sim$ 180° (169° in the crystal) and $\sim$ 0° (this last result being predictable from the millimeter symmetry of the heterocycle).

Cardiovascular effects. After i.v. injection of the compounds studied, there is a short initial increase in rat blood pressure which is low or even negligible with high doses of IPRO-4 and xylazine. This pressor effect is followed by a decrease in blood pressure, and the dose-response curves of maximal hypotension observed are shown in Fig. 6. The most potent drugs, lofexidine and clonidine, induce a 25% decrease in blood pressure at about 6–10 μg/kg.

To obtain similar results with xylazine and tiamenidine, doses ranging from 10 to 300 g/kg are required. However, the hypotension induced by xylazine was higher than that obtained with tiamenidine (between 12% and 20%). IPRO-4 was about 1000 times less active than lofexidine. In order to correlate quantitatively the hypotensive activity of the substances, the doses required to provoke a 25% decrease in mean arterial pressure are reported in Table 3.

For all of the compounds, the hypotensive response was accompanied by a decrease in heart rate frequency. This bradycardic effect, measured at the moment of maximal hypotensive effect, is plotted against the log dose in Fig. 7. The order of potency for this bradycardic effect parallels the decrease in mean arterial pressure. Lofexidine and clonidine are the most potent derivatives. Xylazine and tiamenidine provoked the same bradycardic effect (20% decrease of beats per minute at 100 μg/kg), although xylazine at the same dose was almost twice as hypotensive as tiamenidine (see Fig. 7). With IPRO-4, the bradycardic effect, whatever the dose, was slightly higher than that for the mean arterial pressure.

Intravenous compounds evoked a dose-dependent rise in mean arterial pressure in pithed rats (Fig. 8). Lofexidine, clonidine, and tiamenidine increased mean arterial pressure by +100 mm Hg at doses ranging from 80 to 750 μg/kg. Xylazine and IPRO-4 increased mean arterial pressure less (63–77 mm Hg) at higher doses (3.2 mg and 7.7 mg). The doses (micrograms per kilogram) required to increase the mean arterial pressure by 50 mm Hg are given in Table 3, along with the intrinsic activity of the compounds tested, their pK_a values, and their inhibition constants.

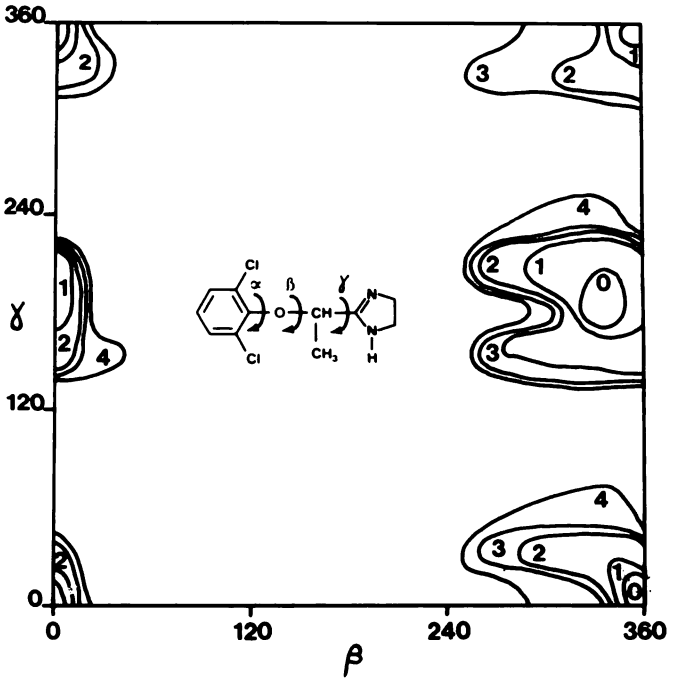


FIG. 5. *Second PCILO conformational energy map of lofexidine with initial values β crystal = 235° and γ crystal = 169°; α crystal = 68° = constant*

Iso-energy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero.

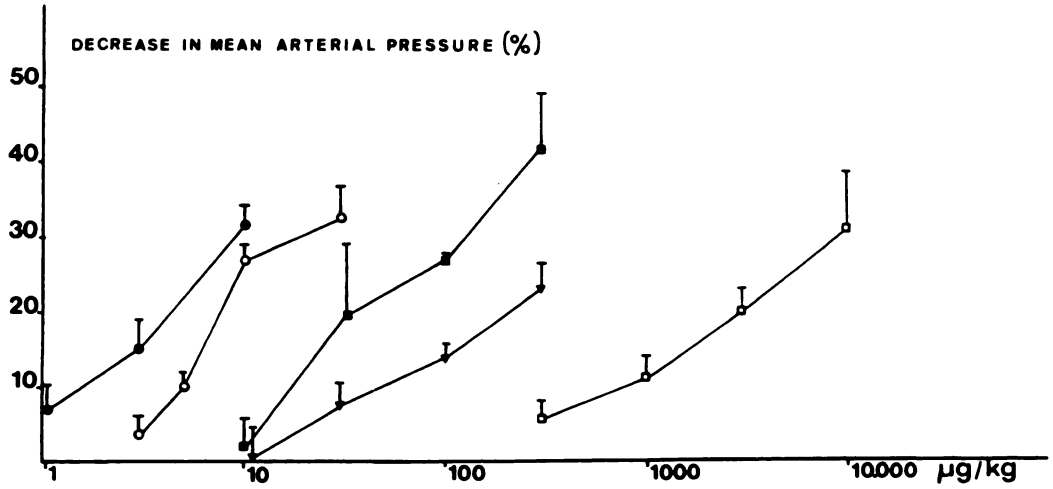


FIG. 6. *Log dose-response curves of the decrease in mean arterial pressure, expressed as percentage of the initial values at the time indicated, following i.v. injection into anesthetized normotensive rats*

Clonidine (○, 30 min), lofexidine (●, 15 min), tiamenidine (▼, 30 min), xylazine (■, 5 min), and IPRO-4 (□, 15 min).

Binding of clonidine analogues to the alpha-adrenergic receptors of rat brain membranes. Our inhibition constants for clonidine, lofexidine, xylazine, and tiamenidine tally with those of Summers *et al.* (31). Clonidine and lofexidine are practically equiactive, and are closely followed in their activities by tiamenidine and xylazine. IPRO-4 is about 100 times less potent than lofexidine. The hypertensive activity of the substances (ED_{50}) is highly correlated with the binding data ($r = 0.99$), but the relationship which exists between the hypotensive activity and the binding ($r = 0.85$) is much less significant. This is understandable, since the central hypotensive activity depends on the ability of the drug to cross the blood-brain barrier.

DISCUSSION

Although the present investigation deals with a limited number of compounds, there are some points of interest. In accordance with the literature (32–35), we found a very good correlation ($r = 0.99$; $n = 5$) between the hypotensive and the bradycardic activity, both of which were obtained by means of i.v. injections into anesthetized normotensive rats. This shows (a) that the pharmacological behavior of all of the drugs is very similar to that of clonidine and (b) that hypotensive and bradycardic effects seem to be mediated by the same mechanism. On the other hand, the link between the hypotensive and the hypertensive activity in pithed normotensive rats is less satisfactory ($r = 0.90$; $n = 5$). This is not surprising, since lipophilicity related to transport processes contributes to the centrally mediated hypotensive activity, whereas this factor is not determining for the peripheral hypertensive effect. Thus, fair correlations ($r = 0.91$) have been obtained between hypotension and a linear combination of hypertension and lipophilicity for a series of 13 homogeneous imidazolidine derivatives (9) Indeed, IPRO-4, which is 80 times less hypertensive than clonidine, is about 550 times less potent as a hypotensive agent, probably because of its unfavorable log P′ value (−0.18 as against 0.93 for clonidine; see Table 3). However, and contrary to rather general opinion, De Jong and Soudijn (36), in a series of cyclic amidines related to clonidine, obtained a good correlation ($r = 0.94$; $n = 8$) between the hypotensive activity and the log of the K_i

TABLE 3

In vitro and in vivo biological data and physicochemical parameters of the clonidine analogues

In vivo results are means of numbers of animals shown in parentheses.

Compound	Hypotensive activity[a]	Bradycardic activity[a]	Hypertensive activity[b]	Intrinsic activity[b]	Binding K_i (nM)[c]	Log P′[d]	pK_a[e]
Lofexidine·HCl	6.2 (18)	5 (18)	5.2 (6)	1.0	2.3	0.47	8.9
Clonidine·HCl	9.5 (23)	8 (23)	7.2 (8)	0.92	2.7	0.93	8.05
Tiamenidine·HCl	300[f] (10)	155 (10)	15 (3)	0.83	4.95	0.48	9.10
Xylazine·H_3PO_4	75 (10)	165 (10)	264 (3)	0.50	120	0.93	7.20
IPRO-4·HCl	5250 (28)	1900 (28)	585 (4)	0.62	218	−0.18	9.16

[a] Hypotensive activity, ED_{25}; bradycardic potency, ED_{25}; i.v. administration to anesthetized normotensive rats.

[b] Hypertensive efficacy, ED_{50}; intrinsic activity (ratio of the maximal increase in blood pressure for each compound to the maximal response to lofexidine; lofexidine = 1), i.v. administration to pithed normotensive rats, expressed as micrograms per kilogram of lofexidine, clonidine, tiamenidine, xylazine, and IPRO-4.

[c] Binding assays on rat cerebral cortex homogenate using [^{3}H]PAC as ligand.

[d] Log of apparent partition coefficient measured between *n*-octanol and aqueous 0.1 M phosphate buffer (pH 7.4).

[e] pK_a values determined in water.

[f] −23% Hypotension.

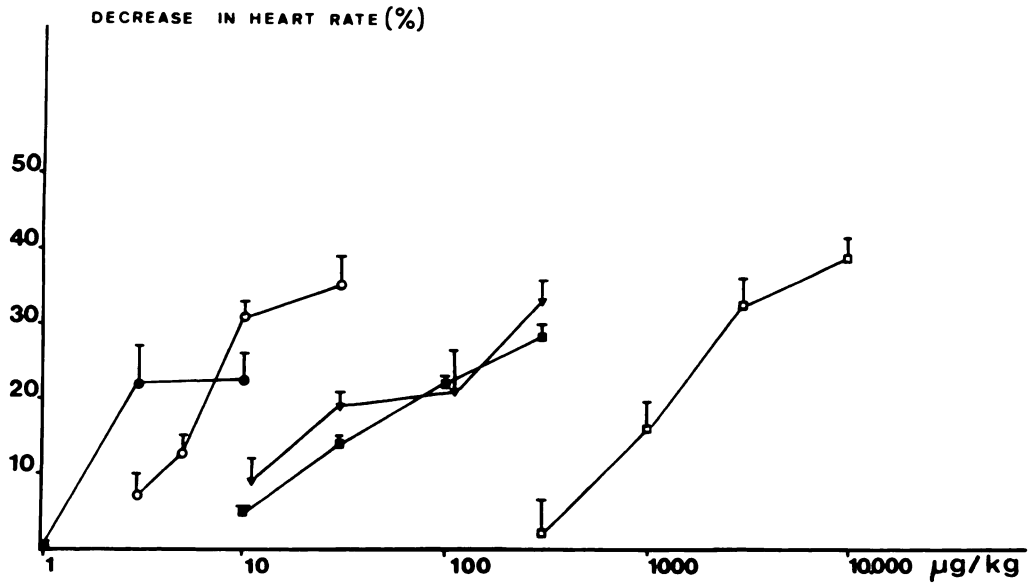


FIG. 7. *Log dose-response curves of clonidine and its analogues with respect to the decrease in heart rate (percentage of initial values) determined at the moment of maximal decrease in mean arterial pressure*

Clonidine (○, 3 min), lofexidine (●, 15 min), tiamenidine (▼, 30 min), xylazine (■, 5 min), and IPRO-4 (□, 15 min).

values, which was not improved when the lipophilic properties of the substances were included. It should be noted, despite satisfactory lipophilicity, tiamenidine and xylazine are weak hypotensive agents. This weak hypotensive activity of xylazine runs with its high inhibition constant (K_i = 120 nM). As expected, and probably for the same reasons, the correlation which exists between the bradycardic effect and the hypertension is limited (r = 0.92).

Binding is strongly linked with the hypertensive activity, as is shown by the high value (r = 0.99) of the correlation coefficient. A similar result was found by Hammer *et al.* (37) with clonidine, BH-T 933, and BH-T 920 between the increase in blood pressure in the pithed rat and the IC_{50} values of [^{3}H]clonidine binding. Since [^{3}H]PAC and [^{3}H]clonidine preferentially label *alpha*$_2$-receptors (20), good correlations observed between blood pressure increase in pithed rat and K_i values for rat cortex membranes suggest that clonidine-like compounds induced hypertension through vascular *alpha*$_2$-adrenoreceptors. This is in agreement with recent findings which show that hypertensive effects in pithed rats in-

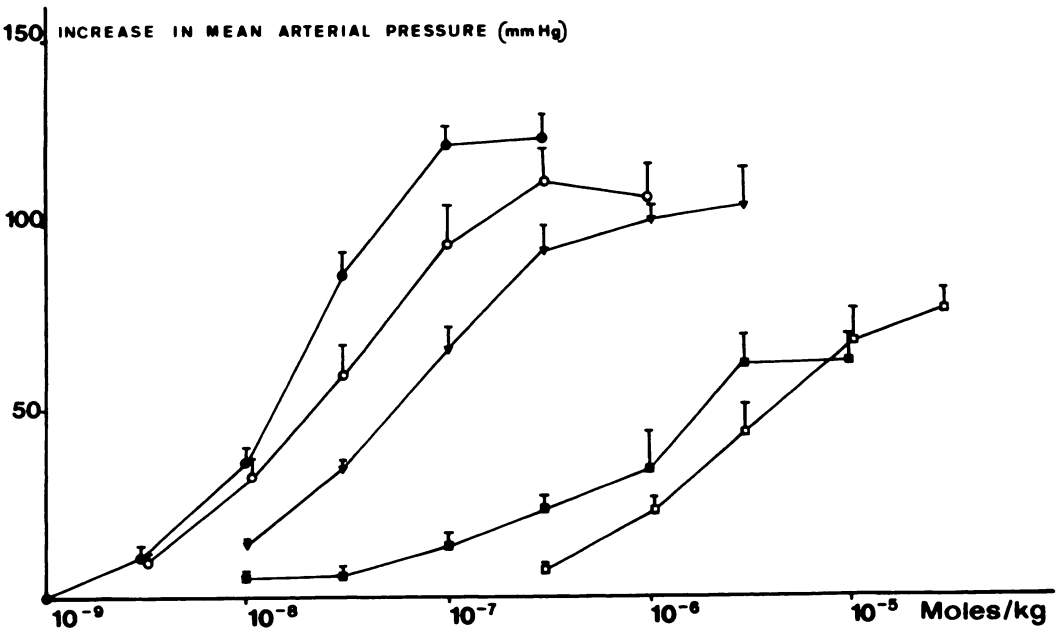


FIG. 8. *Log dose-response curves of the pressor response induced by clonidine and its analogues after i.v. injection into pithed normotensive rats*

Curves are given for clonidine (○), lofexidine (●), tiamenidine (▼), xylazine (■), and IPRO-4 (□). Each point represents the mean ± standard deviation of six to eight determinations.

volved *alpha*$_2$- as well as *alpha*$_1$-postsynaptic adrenoceptors (38).

It has been shown that substitution of the aromatic moiety in aryliminoimidazolidines greatly affects the hypotensive activity (21, 39, 40) and also the *alpha*$_1$- and *alpha*$_2$-adrenergic potency (41). The nature of the function between the aryl and the imidazolidine rings has also been shown to have a strong influence upon the hypotensive potency of some bridged analogues of clonidine (5). It was therefore of particular interest to determine whether the potent activity of lofexidine, as compared with clonidine, could be explained by a close structural analogy. Indeed, X-ray analysis and PCILO calculations presented here clearly demonstrate the striking resemblance between these two *a priori* different molecules.

Crystallographic data have confirmed that, in all of the aryl-iminoimidazolidines base, the C=N double bond is shorter (mean value 1.293 Å) than the C(7)—N(2) or C(7)—N(3) bonds, and consequently the imino form of the molecule predominates. This is in perfect agreement with spectroscopic studies. Upon protonation of the N(1) nitrogen the C—N bond lengths become very similar (mean value 1.325 Å) and lie between a single and a double bond, the positive charge being dispersed over the nitrogen atoms of the imidazolidine group. Each H(N) bears about a 0.17 positive charge, whereas in the case of lofexidine, a small part of this charge is also spread over C(1) and C(11). The electron density distribution (Table 2) shows that, in xylazine and IPRO-4, the positive charge located on H-N(2) is twice (approximately 0.40) as high as in clonidine (0.17) or lofexidine (0.20). This observation might account for an unfavorable interaction at the *alpha*-receptor level.

To illustrate the comparison between the five molecules tested, we have summarized in Table 4 some of their characteristics that emerged from the X-ray analyses and quantum mechanical calculations. Similar results for tolonidine:2-(2-chloro,4-methylphenylamino)-imidazoline nitrate (42) and naphazoline (8) are also presented. The D, H, and ϕ features (Fig. 9) are those we proposed some years ago for clonidine from spectral studies and binding stereomodels (2). Angles ψ and ϕ have slightly different values since they differ in definition: ψ is defined in Fig. 3, and ϕ is the angle between two mean-planes of atoms. D values range from 4.82 to 5.08 Å (mean value 4.95 Å). H values show a greater dispersion; apart from clonidine phosphate, the H range is between 0.72 and 1.04 Å (mean value 0.91 Å). Clonidine phosphate differs from the other protonated molecules, including clonidine hydrochloride, by a higher H value (1.25 Å) and a ϕ value close to 90°. It is then comparable to naphazoline, for which PCILO calculations predict H = 1.33 Å and ϕ = 90° (8). Nonetheless, apart from clonidine phosphate and lofexidine, it seems that, when the aromatic ring is substituted with both chlorine or CH_3 in positions 2 and 6 (or the thienyl ring is substituted in positions 2 and 4), the ϕ angle is close to 75°. In tolonidine, where position 6 of the benzene ring is free, $\phi \sim 60°$. In clonidine and xylazine phosphates and lofexidine, ϕ is close to 90°. It must be remembered that $\phi \sim 60°$ corresponds to the *gauche* conformation and $\phi \sim 90°$ to the *trans* conformation of biological phenethylamines. Recent photoelectron spectroscopic measurements and CNDO/2 quantum mechanical calculations have indicated a torsion angle close to orthogonality even for unsubstituted phenylimino imidazolidine derivatives (43). In any case we suggest that dihedral angles of 60° and 75° are real and important structural features of some protonated forms. Some years ago, Van-Benthem Meerman *et al.* (44), using standard bond length and CNDO/2 calculations, proposed a rather more acute angle, about 40°.

TABLE 4

Geometrical characteristics emerging from X-ray analysis and quantum mechanical calculations

Protonated molecules and salt	ψ°		D (Å)	H (Å)	Φ (°)
	Crystal	Vacuum			
Clonidine					
Hydrochloride	76	74	4.89	1.02	75
Phosphate	86	74	4.82	1.25	89
Xylazine phosphate	81	81	4.84	1.04	81
Tiamenidine hydrochloride	77	73	5.08	0.85	77
IPRO-4 hydrochloride	77	73	5.01	0.82	74
Lofexidine hydrochloride	—	—	5.07	0.72	88
Tolonidine nitrate	53	67	4.93	0.84	55
Naphazoline,[a] base	—	90	5.25	1.33	90

[a] Since the X-ray determination was not made, the figures given were obtained by calculations according to ref. 8.

In the model presented in Fig. 9, the suggested interactions of clonidine-like drugs at *alpha*-adrenoceptors include the following features: (a) an electrostatic attraction between the guanidine function and a negatively charged site of the *alpha*-receptor, (b) a hydrophobic interaction between the aromatic nucleus and an electron-deficient area of the *alpha*-receptor, and (c) the possible additional formation of a hydrogen bond with the exocyclic NH. The results with lofexidine, which is devoid of such a group, clearly confirm that this additional bond is not essential. More important in our opinion are the distances and charge density within the ligand which are required for good interaction at the *alpha*-receptor.

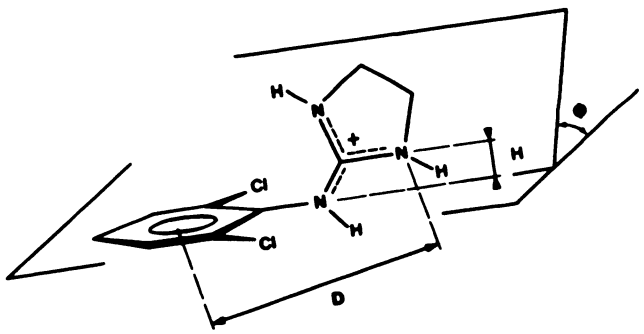


FIG. 9. *Characteristic distances and angle in the postulated model for the interaction of protonated clonidine with the alpha-adrenoceptor*

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