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The effect of 2-substitution on conformations and brain concentrations of phenothiazine-neuroleptics in relation to dopamine-antagonism

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Conformational energies of promazine, chlorpromazine and triflupromazine were calculated with the INDO-method and plotted as a function of two torsion angles. Distance maps were constructed for the N–S and N–2-substituent distances and compared with the N–O distances in dopamine in its preferred conformation, demonstrating the flexibility of the phenothiazine molecules, and showing that interactions with various dopamine-receptor sites, are likely. To eliminate the influence of transport and distribution on the activity, rat brain concentrations of the phenothiazines were determined simultaneously with the increase in striatal homovanillic acid (HVA) concentrations. Concentrations causing a 300% rise in HVA (taken as a measure of neuroleptic activity) were compared with i.p. doses inducing the same rise in HVA, showing that relative potencies based on brain concentrations and relative potencies obtained from i.p. doses were virtually equal. As the increase in lipophilicity does not significantly improve penetration into the brain, it is concluded that the enhancing effect of 2-substitution on the neuroleptic activity might be due to (a) the increase in the probability of conformations in which the side chain is directed towards one side of the molecule and (b) to a direct interaction with the receptor at a site corresponding with one of the dopamine oxygens.

Many investigations indicate that the therapeutic action of phenothiazine drugs involves blockade of pre- and/or postsynaptic dopamine receptor sites. Previously we have shown a correlation between the increase in dopamine turnover in rat striata induced by neuroleptics and their clinical efficacy (Rollema et al 1976). Changes in dopamine turnover were measured in vivo by determination of the concentrations of its major metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC); similar correlations were found using the binding of neuroleptics to striatal homogenates as an in vitro model of the dopamine receptor (Creese et al 1976). From studies with molecular models it was suggested that the interaction between phenothiazines and the dopamine receptor might arise from the similarity between dopamine and part of the chlorpromazine molecule (Horn & Snyder 1971). This idea was evaluated by potential energy calculations which appeared to demonstrate that the molecular conformation of the side chain was determined by van der Waals interactions between the side chain amine and the 2-substituent of the phenothiazine molecule (Feinberg & Snyder 1975).

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Recent reports, however, have questioned this particular role of the 2-substituent as well as the similarity between dopamine and neuroleptics (Tollenaere et al 1977). Thus, it is still uncertain whether the conformation of the phenothiazines as receptor bound species is related to the experimentally measured conformations in the crystal or the aqueous state and what role, if any, the 2-substituent plays in determining this conformation. We now have considered the flexibility of drug and receptor in an attempt to determine the relationship between side chain conformations and the internal energies of the molecules and to establish the consequences for some intramolecular distances.

Conformational energy calculations have previously been performed on the phenothiazine system (Coubeils & Pullman 1972) using the PCILO method. These calculations, however, were made on a model compound with a cationic $-N^+(CH_3)_3$ group. The results showed that the conformation depends on the folding of the rings along the S–N central axis and two equivalent global minima at $\tau_1 = 30°$, $\tau_2 = 60°$ and $\tau_1 = 30°$, $\tau_2 = 180°$ were found. From other calculations on chlorpromazine (Kaufman & Kerman 1972) using the CNDO/2 method it was concluded that the X-ray conformation lies close to a calculated minimum.

Our calculations were performed with the SCF

method at the INDO level of approximation with chlorpromazine as the basic structure for which geometrical data were taken from X-ray data (McDowell 1969). Four torsion angles were considered in our calculations (Fig. 1). When variations of 60° were made in τ_3 and τ_4, with τ_1 and τ_2 fixed at 180°, we found that for all values of τ_4 the lowest total energy values were obtained for $\tau_3 = 180°$. The

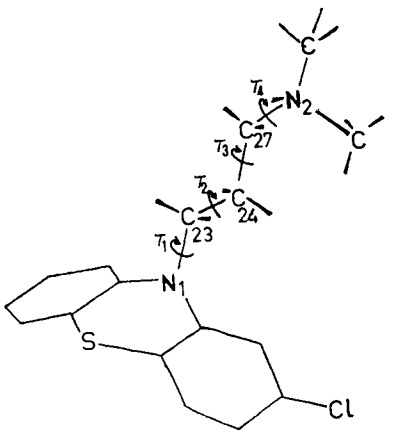

FIG. 1. Structure of chlorpromazine. The four torsion angles are $\tau_1(C_{24}\text{-}C_{23}\text{-}N_1\text{-}S)$, $\tau_2(C_{27}\text{-}C_{24}\text{-}C_{23}\text{-}N_1)$, $\tau_3(N_2\text{-}C_{27}\text{-}C_{24}\text{-}C_{23})$ and $\tau_4(C_{29}\text{-}N_2\text{-}C_{27}\text{-}C_{24})$. The torsion angle (A-B-C-D) between the atoms A-B-C-D represents the angle between the planes ABC and BCD viewed from the direction of A, ABC rotating clockwise.

variations in τ_3 gave energy values which were virtually the same for both $\tau_4 = 120°$ and 180°. We therefore fixed τ_3 and τ_4 at 180° and calculated energies for promazine, chlorpromazine and trifluprazine as a function of the torsion angles τ_1 and τ_2 with increments of 30°. Dependent upon the shape of the individual energy curves giving the variation in one torsion angle with the second held constant, additional refinements were made. A conformational energy map was then constructed with iso-energy curves 1 kcal mole^{-1} (4·185 kJ mol^{-1}) above the global minimum at $\tau_1 = 140°$, $\tau_2 = 60°$ (Fig. 2–4).

The position of the minima on the energy maps indicates a tendency of the side chain to bend to one side of the phenothiazine molecule. Since the same conclusion has been reached for promazine itself, it is difficult to sustain the view that the substituent directs the side chain, by van der Waals or electrostatic forces towards the substituent-bearing benzene nucleus. A similar conclusion was also reached by Horn et al (1975), who found that in the crystal structures the distances involved were too great to

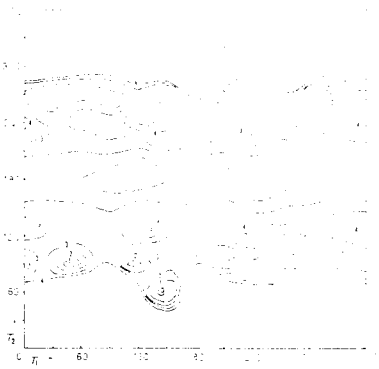

FIG. 2. Conformational energy map for promazine with iso-energy curves 1 kcal mol^{-1} (4·185kJ mol^{-1}) above the global minimum at $\tau_1 = 150$, $\tau_2 = 60$.

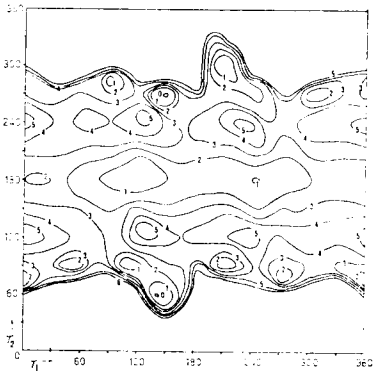

FIG. 3. Conformational energy map for chlorpromazine with iso-energy curves 1 kcal mol^{-1} (4·185kJ mol^{-1}) above the global minimum at $\tau_1 = 150$, $\tau_2 = 270$.

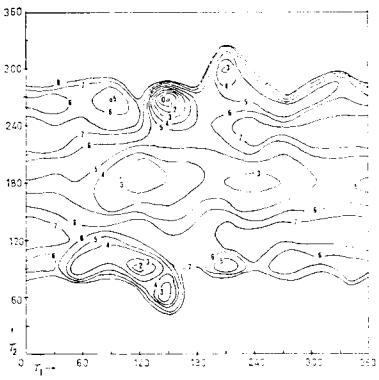

FIG. 4. Conformational energy map for trifluprazine with iso-energy curves 1 kcal mol^{-1} (4·185kJ mol^{-1}) above the global minimum at $\tau_1 = 150$, $\tau_2 = 270$.

allow significant forces of attraction. One effect of the substituent is clearly seen from the energy maps of promazine, chlorpromazine and triflupromazine, namely the differences in size and depth of the energy wells, increasing the probability for assuming the conformations at the energy minima, and hence for the position of the side chain towards the substituted benzene nucleus.

Another potentially important effect of the substituent is its influence on the physicochemical properties of the phenothiazines. Undoubtedly the substituent plays a vital role in determining the lipophilicity of the compounds. This can be shown by a comparison of the apparent partition-coefficients of the compounds, which were determined in octanol-buffer at pH 7·4 (Table 1). As concentrations of a drug in the brain are dependent upon its transport and distribution, differences in these properties, as reflected by the various partition-coefficients, may give rise to differences in brain concentrations and hence to differences in neuroleptic activity of the phenothiazines. To eliminate this influence of the 2-substituent on activity, brain concentrations of the phenothiazines were determined simultaneously with the increase in striatal HVA-concentrations, the latter being a measure of neuroleptic activity. A comparison between brain concentrations and i.p. doses, both of which caused the same rise in HVA, would thus demonstrate to what extent the 2-substituent affects activity by changes in pharmacokinetic properties. We determined the concentrations of promazine, chlorpromazine and triflupromazine in rat brain with a g.c.-m.s. method (Wiesel et al 1975): promazine (40–320 mol kg^{-1}), chlorpromazine (3–20 mol kg^{-1}) and triflupromazine (1–5 μmol kg^{-1}) were given i.p. and after 2 h rats were killed and the brains rapidly removed. Internal standards (chlorpromazine and promazine) were added and after homogenization in 1 M HCl, centrifugation and addition of 10 M NaOH, phenothiazines were extracted with heptane–isoamylalcohol and assayed by selected ion monitoring of the M^+ peaks (Finnigan 3300, column: 3% OV-1, 235 °C). Concentrations were calculated from calibration curves and ranged from 6–80 nmol g^{-1} (promazine), 0·5–20 nmol g^{-1} (chlorpromazine) and 0·1–4 nmol g^{-1} (triflupromazine).

Table 1.

	P	EC 300% HVA (nmol g^{-1})	ED 300% HVA (μmol kg^{-1})
Promazine	252	36 (171)	217 (155)
Chlorpromazine	1903	1·4 (7)	5·8 (4)
Triflupromazine	4194	0·2 (1)	1·4 (1)

P: apparent partition coefficients (pH 7·4—octanol–water).
EC 300% HVA: brain concentrations causing a 300% rise in striatal HVA-concentrations.
ED 300% HVA: i.p. doses causing a 300% rise in striatal HVA-concentrations. Relative potencies are given between brackets.

Simultaneously the elevation of HVA-concentrations in the corpus striatum was measured by a semi-automated fluorimetric method, after isolation of HVA on Sephadex G 10 (Westerink & Korf 1977). From log concentration-response curves we calculated the phenothiazine concentration causing a 300% rise in HVA. When these concentrations, given as ratios with triflupromazine = 1 in Table 1, are compared with ratios of i.p. doses causing the same increase in HVA concentrations, obtained from previously determined log dose-response curves (Rollema et al 1976), it is surprising to see that differences between the two sets of values are quite small. Thus it appears that the phenothiazines are so highly lipophilic that 2-substitution does not contribute significantly to penetration into the brain.

The differences in capability of the 2-substitutents in creating a hydrophobic interaction at the receptor (as exemplified by their different partition-coefficients) remains as a possible explanation for observed differences in potency. The conformational possibility for such an interaction was therefore examined. On the basis of the hypothesis of the resemblance of dopamine to moieties of the phenothiazines we examined crucial distances in both molecules assuming that the phenothiazines and dopamine probably have to share intramolecular geometrical parameters in order to bind to the same receptor. The assumption that dopamine and the neuroleptics bind to the same receptor is supported by the fact that a competitive interaction is found in studies on the influence on DA-sensitive adenylcyclase (Miller et al 1974; Clement-Cormier et al 1974) and in direct receptor binding studies (Seeman et al 1976; Creese et al 1976).

For dopamine we will consider the nitrogen-catechol oxygens distances and for the phenothiazines the distance of the side chain nitrogen to the sulphur atom and to the 2-substitutent. In our model we ignore the distance of the nitrogen to the centre of the aromatic ring, for, in our opinion, the binding of the side chain nitrogen, the oxygen and sulphur atoms and 2-substituents to a receptor will be of more

importance than a fixed position of the aromatic nucleus. It has been shown (Grol & Rollema 1977) that for dopamine the preferred conformations for dopaminergic activity are those around the energy minimum in which the side chain was extended and perpendicular to the aromatic ring. In this region the N–O_a distance might vary between 7·50–7·85 Å and the N–O_b distance between 6·20–7·35 Å (Fig. 5).

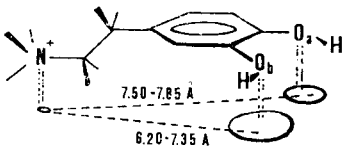

FIG. 5. Dopamine receptor "mapped" by the nitrogen and the catechol oxygens for dopamine in conformations around the energy minimum at $\tau_1 = 90$, $\tau_2 = 180$.

For chlorpromazine, distance maps were constructed giving the N_2–S and the N_2–Cl distances as a function of the two torsion angles τ_1 and τ_2 (Figs 6 and 7). Fig. 6 shows that there is a large area (shaded) in which the N_2–S distance lies between 6·20–7·35 Å, indicating all conformations in which the sulphur atom might interact with a site on the receptor mapped by the O_b atom of dopamine. In a smaller area (crossed in Fig. 6) interaction can also take

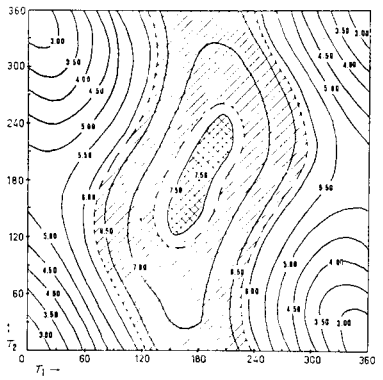

FIG. 6. Isodistance map for chlorpromazine as a function of τ_1 and τ_2, each line giving a fixed value for the N_2-S distance. Areas corresponding to the N-O_a (crossed) and the N-O_b (shaded) distances in dopamine are indicated.

place with the O_a mapped area. The same picture can be developed for the N_2–Cl distance. In two areas the substituent (Cl) might interact with the O_b site and in smaller areas (crossed) with the O_a site (Fig. 7).

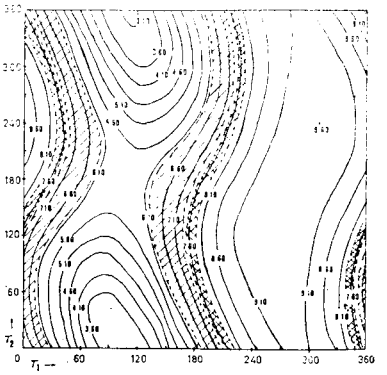

FIG. 7. Isodistance map for chlorpromazine as a function of τ_1 and τ_2, each line giving a fixed value for the N_2-Cl distance. Areas corresponding to the N-O_a (crossed) and the N-O_b (shaded) distances in dopamine are indicated.

All these areas will be energetically restricted if conformations above 5 kcal mol^{-1} (21 kJ mol^{-1}) are considered less probable. This is clearly seen when these areas are transferred onto the energy map (Fig. 8).

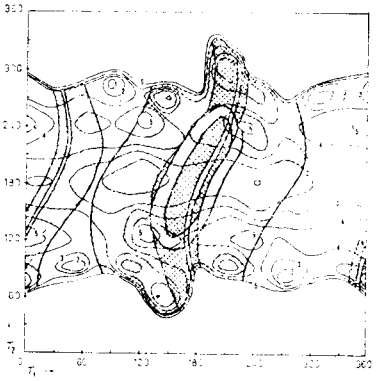

FIG. 8. Energy map for chlorpromazine; N_2-S and N_2-Cl distances corresponding to N-O_a (crossed) and N-O_b (shaded) distances in dopamine are indicated.

From these results it is obvious that the phenothiazines are flexible molecules and that many conformations exist in which either the sulphur atom or the 2-substituent can interact with a dopaminergic receptor at the O_a and O_b mapped sites. Conformations in which both phenothiazine moieties might interact with the catechol areas are also possible. The fact that phenothiazines do interact with various dopamine-receptor sites is not surprising when it is

borne in mind that dopamine-agonists, even semi-rigid analogues such as the 2-amino-1,2,3,4-tetrahydronaphthalenes, interfere with several events involving dopamine, for example release and uptake mechanism (Horn et al 1978) and binding to dopamine-receptor sites (Burt et al 1976).

However, in our opinion it would still be fruitful to synthesize rigid analogues of phenothiazines with fixed interatomic distances, in an attempt to elucidate the structural requirements for the interaction with certain dopamine receptors.

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