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Trifluoperazine is More Effective than Chlorpromazine in Releasing Oxygen from Haemoglobin and Myoglobin

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Abstract

The extent of oxygen release from two heme proteins, haemoglobin and myoglobin have been studied in the presence of trifluoperazine and chlorpromazine (5–1000 μM).

At a molar ratio (drug : protein) of 1·5, the release of oxygen from haemoglobin was 4 and 15% in the presence of chlorpromazine and trifluoperazine respectively, while from myoglobin the corresponding values were 20 and 40%.

The findings were attributed to the greater extent of local conformational change around tryptophan moieties of each of the proteins induced by trifluoperazine.

Chlorpromazine and trifluoperazine are non-planar phenothiazine drugs widely used in the treatment of psychoses. The basic structural differences between the two drugs is that trifluoperazine has an extra hydrophobic group in its tail region and has three fluorine atoms instead of one chlorine atom in chlorpromazine (Fig. 1). We have already reported that chlorpromazine binds to tetrameric haemoglobin in an electrostatic and cooperative manner which is in sharp contrast with its hydrophobic and non-cooperative mode of binding with monomeric myoglobin (Bhattacharyya et al 1994). Analogous binding behaviour has also been obtained with trifluoperazine binding to haemoglobin and myoglobin (unpublished results).

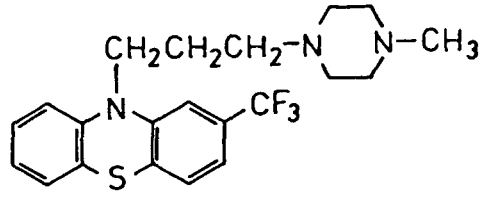
Hele (1964) pointed out that phenothiazines are surface-active agents and their surface activity is related to their potency as tranquillizers with trifluoperazine being more potent therapeutically than chlorpromazine.

Here we report the difference in the extents of oxygen release from haemoglobin and myoglobin due to the interaction with trifluoperazine and chlorpromazine.

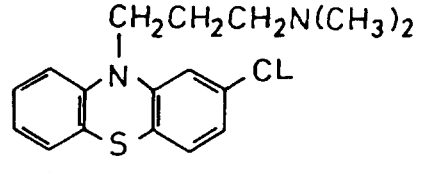
Materials and Methods

Chlorpromazine and trifluoperazine were obtained as gifts from Sun Pharmaceuticals, India. Myoglobin was purchased from Sigma Chemical Co., USA. Oxymyoglobin was prepared from the purchased stock by the method of Dixon & McIntosh (1967). Other chemicals used in the experiments were of analytical grade. Aqueous stock solutions of chlorpromazine and trifluoperazine were made before each set of experiments. The concentration of the drug solutions were determined spectrophotometrically from their respective molar extinction coefficients (chlorpromazine 305 nm, 4000 M^{-1} cm^{-1}; trifluoperazine 308 nm, 3162 M^{-1} cm^{-1}).

Tetrameric haemoglobin was isolated and purified from human blood donated by healthy non-smoking male volunteers, aged 22–25 (Bhattacharya et al 1990). Stock concentrations of either haemoglobin or myoglobin in phosphate buffered saline (PBS, 0·15 M NaCl, 0·002 M sodium phosphate, pH 6·8) were determined from their Soret absorbances with molar extinction coefficients at 415 nm of 125 mM^{-1} cm^{-1} and at 418 nm of 128 mM^{-1} cm^{-1}, respectively. The oxygen content of both oxyhaemoglobin and oxymyoglobin estimated from the characteristic absorption spectra of each of the proteins was found to be 100%. All fluorescence measurements were performed in a Hitachi F3010 spectrofluorometer using a 1-cm path-length cuvette as described previously (Bhattacharyya et al 1994). Titration of the drug-bound-protein was done from the quenching of



Trifluoperazine



Chlorpromazine

FIG. 1. Structures of chlorpromazine and trifluoperazine.

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protein fluorescence at 332 nm when excited at 285 nm by successive addition of the drug from a stock solution to 3 mL 8 μM protein.

Oxygen release from haemoglobin and myoglobin was measured in a Gilson 5/6 oxygraph machine. The change in partial pressure due to released oxygen in the haemoglobin or myoglobin solution in a stoppered cell was detected by the membrane-covered oxygen electrode fitted with the cell. The output signal was recorded in the oxygraph chart as a function of time. PBS alone showed no change in the output signal even after 30 min stirring. The amount of free dissolved oxygen in PBS was taken to be 250 nmol mL^{-1} (West 1985). Calibration of the oxygraph chart in terms of the nmol oxygen release was from the change in the output signal due to the total depletion of free oxygen from 2 mL buffer when 0·1 g sodium metabisulphite was added. No oxygen was found to be released from tetrameric oxyhaemoglobin or monomeric oxymyoglobin in 0·15 M NaCl, pH 6·8 in the absence of the drug. The temperature during the experiment was maintained at 27°C. Each data point in Figs 3 and 4 represents the mean of five individual experiments and test of significance of the data corresponding to each ratio was by means of a t-test.

Results and Discussion

Fig. 2 is the representative plot in the oxygraph chart for the release of oxygen from a fixed concentration of haemoglobin (200 μM) due to the gradual addition of trifluoperazine. The rate was high immediately after the addition of the drug followed by a relatively slower rate. Similar patterns of oxygen release were also observed for haemoglobin-chlorpromazine, myoglobin-chlorpromazine and myoglobin-trifluoperazine. However, the rate of oxygen release was greater for haemoglobin-trifluoperazine and myoglobin-trifluoperazine than for haemoglobin-chlorpromazine and myoglobin-chlorpromazine, respectively.

Fig. 3 shows the release of oxygen from oxyhaemoglobin and oxymyoglobin as a function of molar ratio of drug: protein, and shows that the percentage of oxygen release from both haemoglobin and myoglobin after a fixed interval of time (2 min) increases with the increase in the stoichiometric ratio of drug : protein. Since the amount of drug is higher at a higher ratio, the extent of saturation of the drug-protein binding is greater at higher ratio. Dependence of the release of oxygen on the ratio suggests that oxygen release is related to the extent of saturation of the drug-bound proteins. From Fig. 3 it is evident that at the same ratio, trifluoperazine is more efficient with respect to extent of oxygen release from either protein. For example, at a molar ratio of drug : protein of 1·5, the release of oxygen from oxyhaemoglobin was 4 and 15% in the presence of chlorpromazine and trifluoperazine, respectively, while for oxymyoglobin, the corresponding values were 20 and 40%. The percentage of oxygen release from haemoglobin due to drug binding was cooperative.

Fig. 4 shows the emission spectra of the complexes. The change in E_{max} was measured with respect to the emission maximum of untreated haemoglobin or myoglobin. Addition of either drug led to an increase in the wavelength of the emission maximum (red shift) of both proteins as a function of the increase in the added drug concentrations. Therefore, concomitant with the drug-protein interactions, a change in the conformation of these two proteins always occurs in such a way that the tryptophan moieties of the protein molecules are more exposed to the polar region. We have already shown that tryptophan residues are in or near the possible binding sites for haemoglobin and myoglobin interacting with chlorpromazine (Bhattacharyya et al 1994) and trifluoperazine (unpublished results). It is evident from Fig. 4 that upon binding of the phenothiazine, haemoglobin exhibits greater cooperativity in

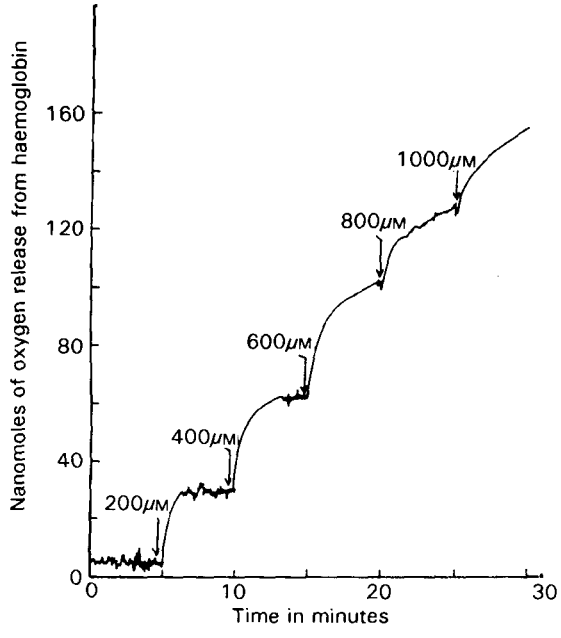

FIG. 2. Gilson 5/6 oxygraph chart of the release of oxygen from 2 mL haemoglobin (200 μM, monomer basis) solution in 0·15 M phosphate-buffered saline, pH 6·8, when trifluoperazine at different final concentration was added at different times (arrows).

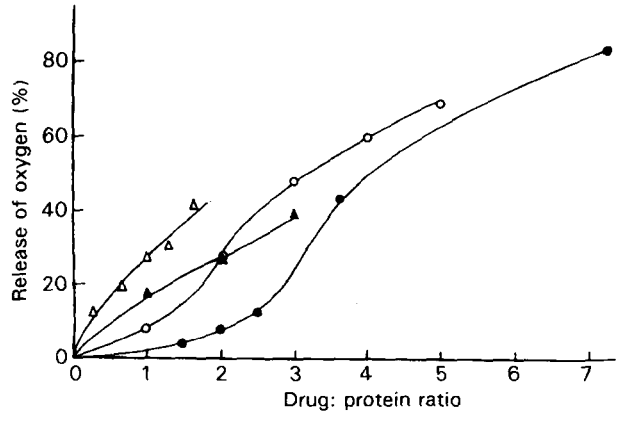

FIG. 3. Release of oxygen from oxyhaemoglobin or oxymyoglobin as a function of molar ratio of drug : protein. Percent release of oxygen was estimated from nmol oxygen released from 2 mL oxyhaemoglobin (monomer basis) or oxymyoglobin in phosphate-buffered saline (0·002 M phosphate, 0·15 M NaCl, pH 6·8) in the presence of the drug in the oxygraph. Before addition of drug both haemoglobin and myoglobin in the oxygraph cell were taken to be 100% oxygenated. The drug : protein ratio was varied either by varying the drug concentration for a fixed concentration of the protein or by changing the protein concentration for a fixed drug concentration. ● Haemoglobin-chlorpromazine; ○ haemoglobin-trifluoperazine; ▲ myoglobin-chlorpromazine; △ myoglobin-trifluoperazine.

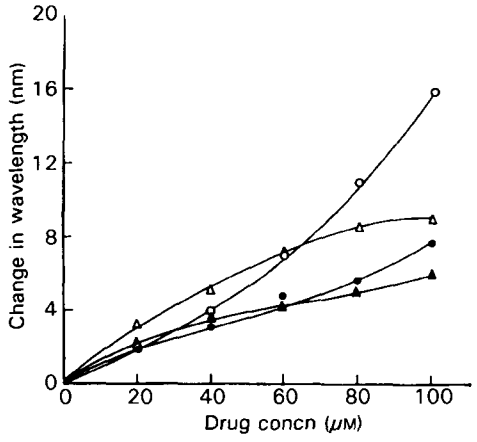

FIG. 4. Change in emission maximum wavelength of haemoglobin or myoglobin in the presence of the drug. The change in emission maximum wavelength was measured with respect to the emission maximum of the corresponding protein in the absence of the drug. (●) Haemoglobin-chlorpromazine; ○ haemoglobin-trifluoperazine; ▲ myoglobin-chlorpromazine; △ myoglobin-trifluoperazine.

the conformational change than does myoglobin. Here it is worthwhile to mention that both drugs bind to haemoglobin in a cooperative manner in comparison with the binding to myoglobin which is non-cooperative (Bhattacharyya et al 1994). It is also clear that trifluoperazine-induced conformational change of either protein is greater than that of the chlorpromazine-induced change. It has also been found from the circular dichroism spectra that the secondary structural organization of both proteins as estimated by the α-helix content (75% -helix) does not alter appreciably in the presence of the drug (data not shown).

Thus interaction of phenothiazine drugs, chlorpromazine and trifluoperazine, with haemoglobin and myoglobin bring about only local conformational change around tryptophan moieties in the proteins as well as leading to the release of oxygen from the proteins. These drug-induced effects are probably related, as trifluoperazine is more effective than chlorpromazine in causing both effects. The structural differences between chlorpromazine and trifluoperazine may be responsible for the observed drug-induced effects. However, the oxygen release from haemoglobin and myoglobin by phenothiazines that we have reported here might be an adverse effect which should therefore be given due consideration in prescribing or administering phenothiazines.

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