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ANTIHEMOLYTIC ACTIONS OF TRICYCLIC TRANQUILIZERS

STRUCTURAL CORRELATIONS*

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Abstract—Thirty-one phenothiazines were examined for their capacity to protect red blood cells of the dog against hypo-osmotic hemolysis. Incubation of erythrocytes in buffered saline (135 mOsmoles/l.) resulted in 40–50 per cent hemolysis. Addition of phenothiazines to this system generally reduced hemolysis. The 50 per cent effective dose (ED_{50}) was determined for each compound. The ED_{50} ranged from 0·002 to 0·043 mM. Protection against hemolysis was related to molecular structural features: substitutions at position 10 showed no predictable influence on hemolysis; two sulfoxides had no protective influence; protection increased with increasing electronegativity at position 2—CF_3 > Cl > H. Imipramine and desmethylimipramine also had antihemolytic activity, but the response was maximal at the smallest dose used and did not change with larger doses. In several experiments, phenothiazines were preincubated with compounds which might be constituents of the red cell membrane. Binding could not be observed between phenothiazines and lecithin or cholesterol. Extensive binding to bovine albumin was demonstrated.

MANY actions of the phenothiazine tranquilizers can be accounted for by their influences either on cellular energy metabolism or on cell membrane permeability.[1] The red blood cell is a familiar model for cell membranes and has been used as a test system for phenothiazine effects. Protection of red blood cells against hypo-osmotic hemolysis by phenothiazines has been demonstrated by several authors in red blood cells from man,[2–4] cow,[4] dog,[5] pig[6] and rat.[7] Other agents,[7] such as local anesthetics,[8] antihistaminics[2] and anti-inflammatory agents,[5] also protect against hemolysis. These effects have been applied to preservation of stored blood[9,10] and to prevention of sickling in subjects with the sickle cell trait.[11] In the following study, 31 phenothiazines were compared for their relative capacities to protect against hemolysis in order to correlate molecular structures with biological activities.

METHODS

One male mongrel dog was used as a blood donor for experiments in which phenothiazines were compared for potency. Additional experiments were performed with blood from at least three other dogs, after first confirming that sensitivity to phenothiazines was equivalent to earlier experiments. Fresh heparinized blood was oxygenated with a mixture of oxygen (90 per cent) and carbon dioxide (10 per cent) for 60 sec.

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The blood was not washed, since washing has a negligible influence on the results.[2] Fifty μl of whole blood was added to 5 ml of a buffered saline solution (0·425 per cent NaCl; sodium-phosphate buffer, 5 mM at pH 7·4; total osmolality 135 mOsmoles/l.). After a single inversion of the tubes to mix the contents, the suspensions were kept at room temperature (22–24°) for 5 min and then were centrifuged for 5 min to separate cells. Hemolysis in these tubes ranged from 40 to 60 per cent and was used as a reference against which to compare the effects of phenothiazines. Drugs were incorporated in the buffered saline solutions before addition of whole blood to yield concentrations of 4×10^{-6}, 10^{-5} and 2×10^{-5} M. After incubation and centrifugation of the hemolyzed cells, the supernatant solutions were diluted with an equal volume of ammoniated distilled water and the optical density of the solutions at 540 mμ was observed in a Coleman Jr. spectrophotometer. Each experiment consisted of triplicate observations on hemolysis with and without drug and was repeated on at least three separate occasions.

In binding experiments, cholesterol, lecithin or bovine albumin (0·5 g/100 ml) was combined with phenothiazine in the buffered saline solutions before addition of red blood cells. Cholesterol (1·0 mM) or lecithin (250 mg/ml) was dissolved in chloroform and 0·1 ml was added to each of several test tubes. Chlorpromazine or trifluopromazine dissolved in chloroform (0·1 ml, 1·0 mM) was added and the mixture was evaporated to dryness. To each of these tubes, buffered saline and red blood cells were added as in previous experiments.

Cooperation of the pharmaceutical industry in supplying generous quantities of drugs is gratefully acknowledged. Specific sources of each drug are listed in Table 1.

RESULTS

Hemolysis of red blood cells in buffered saline solutions having an osmolality of 135 mOsmoles/l. was approximately 50 per cent. Addition of phenothiazine to the incubation media reduced hemolysis appreciably in most instances, and the magnitude of response varied with the dose of drug. Representative results are shown in Fig. 1. From the relationship between dose of drug and relative hemolytic response, it is possible to determine either by extrapolation or by interpolation the concentration of each phenothiazine which reduced relative hemolysis to one-half the value of control observations (ED_{50}). These values are recorded in Table 1.

Similar experiments were performed with imipramine and with desmethylimipramine (Fig. 1). Although both compounds protected against hemolysis, the form of the dose–response curve differed from that observed with the phenothiazines. Instead of producing increasing protection as the dose was increased, maximum protection was achieved with the smallest dose tested. Further increases in concentration did not alter the results.

Attempts were made to demonstrate binding of phenothiazines with cholesterol, lecithin or bovine albumin. These compounds were chosen to represent various components of the cell membrane. Each compound was incorporated in the medium with phenothiazine before addition of red blood cells. If the phenothiazine associated preferentially with the added compound rather than with red blood cells, its effectiveness as an antihemolytic agent should be reduced. Cholesterol or lecithin had no

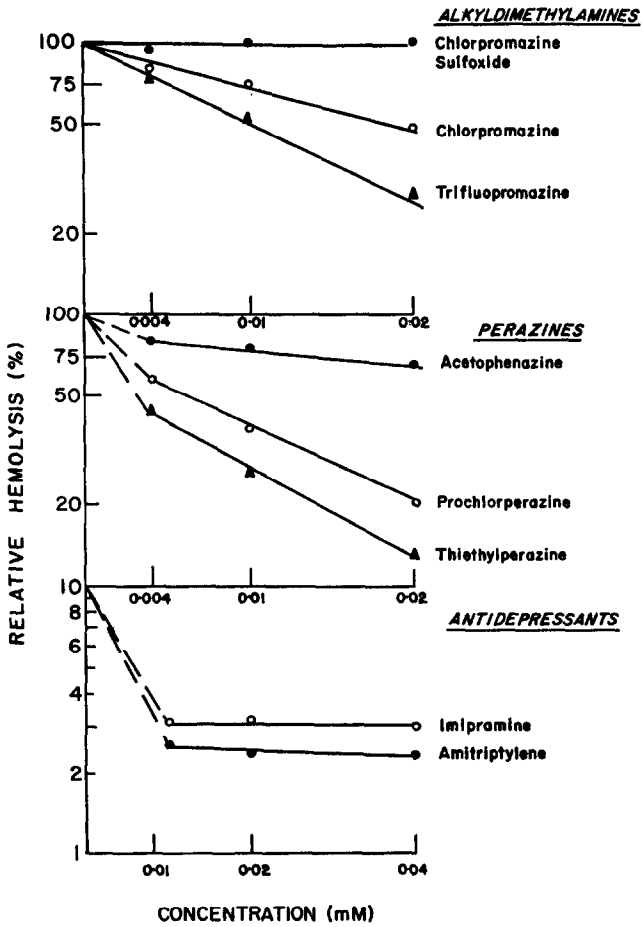


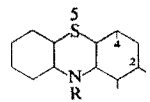
FIG. 1. Representative dose–response curves of phenothiazines and other drugs.

effect on the results. In contrast, bovine albumin completely nullified the protective action of phenothiazines.

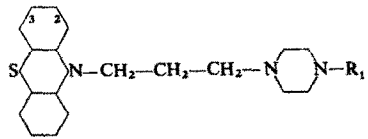
DISCUSSION

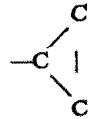
It is now firmly established that phenothiazines protect red blood cells of several animal species against hypo-osmotic hemolysis. Concentrations of phenothiazines required for such an influence are small, in the range from 10^{-8} to 10^{-4} M. At higher concentrations, they are hemolytic.[12] The protective action appears to be an effect on the membrane by which the phenothiazine probably interacts with membrane components. Accumulation of chlorpromazine by red cell ghosts can yield a concentration in membranes which is 3000 times greater than that in the surrounding medium.[2] It has been suggested that absorption occurs at hydrophobic loci.[13,14] Since phenothiazines do not appear to interact either with cholesterol or with lecithin *in vitro*, it is possible that such interaction also does not occur in the cell in spite of

TABLE 1. ANTIHEMOLYTIC POTENCY OF PHENOTHIAZINES



Alkyldimethylamines	50% Effective dose	Substituents 2—	Substituents 10—	Others	Source*
1. Abbot 2665	Indeterminate	—H	—C:O—O—$(CH_2)_2$—N—$(CH_2)_2$		Abb
2. Abbot 2780	Indeterminate	—H	—$(CH_2)_2$—N—$(CH_3)_2$		Abb
3. Chlorpromazine sulfoxide	Indeterminate	—Cl	—$(CH_2)_3$—N—$(CH_3)_2$	5—S → O	SKF
4. SKF 5418-A	Indeterminate	—H	—$(CH_2)_3$—N—$(CH_3)_2$	5—S → O	SKF
5. Prothipendyl	$3{\cdot}1 \times 10^{-5}$†	—H	—$(CH_2)_3$—N—$(CH_3)_2$	N in ring at 1	Abb
6. Acepromazine	$3{\cdot}0 \times 10^{-5}$†	—C:O—CH_3	—$(CH_2)_3$—N—$(CH_3)_2$		Abb
7. Methoxypromazine	$2{\cdot}8 \times 10^{-5}$	—O—CH_3	—$(CH_2)_3$—N—$(CH_3)_2$		Abb
8. Promazine	$2{\cdot}8 \times 10^{-5}$†	—H	—$(CH_2)_3$—N—$(CH_3)_2$		Abb
9. Propiomazine	$2{\cdot}4 \times 10^{-5}$†	—C:O—CH_2—CH_2	—$CH(CH_2)$—CH_2—N—$(CH_3)_2$		Abb
10. Chlorpromazine	$1{\cdot}9 \times 10^{-5}$	—Cl	—$(CH_2)_3$—N—$(CH_3)_2$		SKF
11. Propiopromazine	$1{\cdot}7 \times 10^{-5}$	—C:O—CH_2—CH_2	—$(CH_2)_3$—N—$(CH_3)_2$		Abb
12. Methotrimeprazone	$1{\cdot}6 \times 10^{-5}$	—O—CH_3	—CH_2—$CH(CH_2)$—CH_2—N—$(CH_3)_2$		Abb
13. Chlorprothixene	$1{\cdot}1 \times 10^{-5}$	—Cl	—$(CH_2)_2$—N—$(CH_3)_2$	C in ring at 10	HLR
14. Triflupromazine	$1{\cdot}1 \times 10^{-5}$	—CF_3	—$(CH_2)_3$—N—$(CH_3)_2$		Abb
15. SQ-4757	$8{\cdot}0 \times 10^{-6}$	—H	—$(CH_2)_3$—N—$(CH_3)_2$	4—CF_3	Sq
16. SQ-8914	$6{\cdot}0 \times 10^{-6}$	—CF_3	—$(CH_2)_3$—N—$(CH_3)_2$	7 Cl	Sq

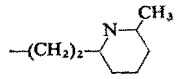
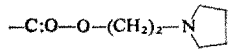
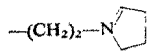
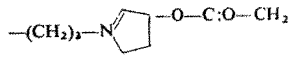
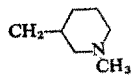
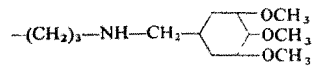
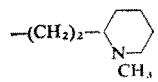


	Perazines	50% Effective dose	Substituents 2—	Substituents —R_1	Others	Source
17.	Acetophenazine	$3{\cdot}6 \times 10^{-5}$†	—C:O—CH_2	—CH_2—CH_2OH		Abb
18.	Perphenazine	$1{\cdot}5 \times 10^{-5}$	—Cl	—CH_2—CH_2OH		Sch
19.	Carphenazine	$1{\cdot}2 \times 10^{-5}$	—$(CH_2)_2CH_3$	—CH_2—CH_2OH		Abb
20.	Fluphenazine	$1{\cdot}0 \times 10^{-5}$	—CF_3	—CH_2—CH_2OH		Abb
21.	Cyclophenazine	$9{\cdot}0 \times 10^{-6}$	—CF_3			Lil
22.	Prochlorperazine	$6{\cdot}0 \times 10^{-6}$	—Cl	—CH_3		Abb
23.	Trifluoperazine	$6{\cdot}0 \times 10^{-6}$	—CF_3	—CH_3		Abb
24.	Thiethylperazine	$2{\cdot}5 \times 10^{-6}$	—CF_3 +	—S—CH_2—CH_3		San

* Drugs were obtained from the following sources: Abb (Abbott); SKF (Smith, Kline and French); (HLR Hoffman-La Roche); Sq (Squibb); Sch (Schering); Lil (Lilly); San (Sandoz).

† Values were determined by extrapolation.

TABLE 1. *cont.*

Others	50% Effective dose	Substituents 2—	Substituents 10—	Substituents Others	Source
25. TPN-12	Indeterminate	—H		3—SO_2—CH_3	San
26. Abbot 3658	$4{\cdot}3 \times 10^{-5}$†	—H			Abb
27. Pyrathiazine	$2{\cdot}2 \times 10^{-5}$†	—H			Abb
28. Abbot 14542	$1{\cdot}6 \times 10^{-5}$	—H			Abb
29. Mepazine	$1{\cdot}5 \times 10^{-5}$	—H			Abb
30. Abbott 17599	$6{\cdot}0 \times 10^{-6}$	—H			Abb
31. Thioridazine	$5{\cdot}0 \times 10^{-6}$	—S—CH_3			Abb

* Drugs were obtained from the following sources: Abb (Abbot); SKF (Smith, Kline and French); HLR (Hoffman–La Roche); Sq (Squibb); Sch (Schering); Lil (Lilly); San (Sandoz).

† Values were determined by extrapolation.

high lipoid solubilities of these compounds. Protein binding of these drugs, on the other hand, certainly does occur, as is supported by results with albumin and by binding of chlorpromazine to hemoglobin.[13] Interaction with sialic acids was not examined in the present studies.

Interaction with red blood cell membranes appears to involve a structural change as seen by light microscopy.[3] Experiments with red cell ghosts demonstrate a 7–10 per cent increase in surface area under the influence of chlorpromazine.[13] Such influence is consistent with the capacity of phenothiazines to lower the surface tension of aqueous solutions.[15]

Calcium also has a place in the understanding of phenothiazine actions, since calcium influences both excitability and permeability of cell membranes. Chlorpromazine can displace calcium from phospholipid monolayers[16] and from red cell ghosts.[13] Other anticholinergic psychoactive drugs can increase rigidity of lipid monolayers,[17] as does calcium. On these grounds, it might be suggested as a basis for further experimentation that phenothiazines exert their influences on membranes by interacting with membrane components at sites normally occupied by calcium. At low concentrations, the membrane becomes more rigid, in spite of a small expansion of surface area. At larger concentrations, the dispersing effect of phenothiazines should dominate, leading to lysis of red cells and perhaps of other intracellular organelles. Perhaps such activity accounts for inhibitory influences on membrane-located enzymes such as adenosine triphosphatases.

In the present series of 31 compounds, several correlations can be made between molecular structure and antihemolytic activity. Sulfoxides (compounds 3 and 4) have no apparent protective effect at the doses tested, although their parent compounds (compounds 8 and 10) are effective. The nature of the constituent at position 10 seems not to have any predictable influence on antihemolytic potency. Comparison of alkyldimethylamines with perazines shows similar potencies for compounds 10 and 18, and for 14 and 20, which differ only in their 10-substituents. Furthermore, the ring nitrogen at position 10 is not significant, since replacement by carbon has no influence on potency (cf. compounds 10 and 13). In each series, there is a correlation between the nature of substituents on the tricyclic nucleus and effectiveness as an antihemolytic agent. When position 2 is unsubstituted, the compounds tend to have less effectiveness, but potency increases with chloro substitution and is highest with a trifluoromethyl substituent. Compound 15 has a high potency in spite of a 2-H constituent, possibly because of its 4-CF_3 group. In a general sense, a similar correlation can be made between halogen substituents and clinical effectiveness as tranquilizing agents.

One might conclude that contact between drug and membrane takes place at the tricyclic nucleus, since the 10-substituent tail appears not to condition the interaction significantly. Further, because of the orientation of the tail, contact should orient the 10-substituent outward from the membrane where it remains free to take part in additional supplementary attractions. The question may be raised whether the halogen substituent on the nucleus takes part in the interaction directly or whether it affects reactivity of the entire nucleus by influencing electron densities at critical loci, possibly positions 5 and 10. At any rate, the high lipoid solubilities of these compounds[12] and the expansion of surface area which they cause[13] suggest that a rearrangement of surface structure takes place, quite possibly at lipophilic loci in the membrane.

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