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SHORT COMMUNICATIONS

Some relationships concerning the chemical structure, hemolytic activity, and therapeutic potency of phenothiazines

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THE ability of phenothiazines to affect cellular[1] and subcellular[2] membranes has been shown to occur in many biological systems and to influence permeability to proteins, ions, and neurohormones. Guth and Spirtes[3] reviewed this literature extensively and proposed that changes in membrane permeability may be the major underlying mechanism in phenothiazine tranquilization.

The present study compares the ability of several phenothiazine tranquilizers to affect cellular permeability. Lysis of sheep erythrocytes was used as a test system, and attempts were made to elucidate the relation of hemolytic potency to the chemical structure and clinical potency of these compounds.

METHODS

Sheep red blood cells were washed with physiological saline and the number in a unit volume standardized. Each chemical was dissolved in physiological saline, adjusted to pH 7·3 to 7·4 with Na_2HPO_4. Since the compounds are not very soluble in saline and form colloid-like substances after standing for a short period of time, 5 mM was the highest concentration used. Different concentrations (5, 2·5, 1·25 mM, etc.) were incubated at 37° with a known amount of washed sheep cells. Saline was then added, the mixture centrifuged, and the optical density of the supernatant measured at 541 mμ. The per cent of cells lysed was determined by comparison with completely lysed cells in a control tube. The data in Table 1 are presented as the concentration of phenothiazine needed to obtain 70 per cent or more lysis of cells. The hemolytic activity of phenothiazines was found to be closely reproducible with a single lot of cells but to vary at times with different lots of cells. The data, therefore, are averages based on three or more experiments.

The partition coefficient is the ratio of the amount in the chloroform layer to the amount in the water layer. Solutions of the phenothiazines ($1{\cdot}25$ to $2{\cdot}5 \times 10^{-5}$ M) were prepared in 0·1 M phosphate buffer (pH 7·4) or in 0·1 N HCl and shaken for 1 hr with an equal volume of acid-washed chloroform; the amount in each layer was determined spectrophotometrically at the absorption maximum (255 to 262 mμ).

The range of daily doses recommended in the Physicians Desk Reference for the treatment of hospitalized psychiatric patients has been used for comparing the therapeutic potencies of the phenothiazines.

Stromata were prepared with washed sheep erythrocytes essentially according to standard procedures.[4] Varying amounts of stromata or hemoglobin were mixed with chlorpromazine (0·2–1·25 mM) in physiological saline, pH 7·4. After incubation at 37° for 20 min, washed sheep erythrocytes were added. Binding of chlorpromazine was considered to be proportional to the reduction in the per cent of cells lysed,[5] as compared to controls with no stromata or hemoglobin added.

The phenothiazines used were obtained through the generosity of the following companies: chlorpromazine (Thorazine), prochlorperazine (Compazine), and trifluoperazine (Stelazine) from Smith, Kline & French Laboratories; fluphenazine (Permitil) from White Laboratories, Inc.; methotrimeprazine (Veractil) and methoxypromazine (Tenton) from Lederle Laboratories; promazine (Sparine) from Wyeth Laboratories; thiopropazate (Dartal) from G. D. Searle and Co.; trifluopromazine (Vesprine) from E. R. Squibb and Sons; and thioridazine (Mellaril) and NP-207 from Sandoz Pharmaceuticals.

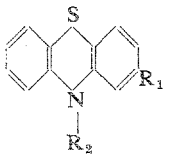
RESULTS

In Table 1 are presented the structures, partition coefficients ($CHCl_3$/0·1 N HCl), average ranges of daily dose in psychiatric patients, and hemolytic potencies of the eleven phenothiazines studied. The compounds are listed in the order of their partition coefficients.

Structure–activity relationships

The data in Table 1 indicate that the structure of either the R_1 or R_2 group may have a strong influence on hemolytic and therapeutic potencies. The contribution of the R_2 group, however, appears to be more frequently important; e.g. the presence of the substituted piperazine group in the R_2 chain markedly enhances hemolytic and therapeutic potencies. A large change in the hemolytic activity may also be caused by a relatively small change in the R_2 chain: methotrimeprazine differs from methoxypromazine only by having a branched methyl group in its R_2 chain, but it is much less hemolytic than the latter compound.

TABLE 1. HEMOLYTIC ACTIVITY, THERAPEUTIC POTENCY, AND PARTITION COEFFICIENT ($CHCl_3$/0·1 N HCl) OF PHENOTHIAZINES



Generic name	R_1	R_2	Hemolytic level (mM)*	Psychiatric dose (mg)	Partition coefficient
Piperidine group					
Thioridazine	SCH_3	$-CH_2CH_2-C_5H_9N-CH_3$	none	200–400	200·0
NP-207 (Sandoz)	Cl	$-CH_2CH_2-C_5H_9N-CH_3$	1·25	200–400	72·0
Promazine group					
Triflupromazine	CF_3	$-CH_2CH_2CH_2-N(CH_3)_2$	1·25	50–150	28·5
Chlorpromazine	Cl	$-CH_2CH_2CH_2-N(CH_3)_2$	1·25	200–500	16·7
Methotrimeprazine	OCH_3	$-CH_2CH(CH_3)CH_2N(CH_3)_2$	none		11·0
Methoxypromazine	OCH_3	$-CH_2CH_2CH_2-N(CH_3)_2$	1·25	200–300	9·4
Promazine	H	$-CH_2CH_2CH_2-N(CH_3)_2$	1·25	200–500	5·4
Piperazine group					
Thiopropazate	Cl	$(CH_2)_3-C_4H_8N_2-CH_2CH_2COOCH_3$	0·5	20–30	4·7
Trifluoperazine	CF_3	$(CH_2)_3-C_4H_8N_2-CH_3$	0·25	10–20	0·7
Prochlorperazine	Cl	$(CH_2)_3-C_4H_8N_2-CH_3$	0·24	30–100	0·4
Fluphenazine	CF_3	$(CH_2)_3-C_4H_8N_2-CH_2CH_2OH$	0·05	0·5–1	0·1

* Concentration that lyses 70 per cent of cells; "none" indicates no hemolysis at 5 mM.

The nature of the R_1 substituent did not appear to have as great an effect on hemolytic potency as it has on clinical potency. Replacement of the chloro group by a trifluoro group greatly enhances clinical potency but did not appreciably alter hemolytic activity in either the promazine or piperazine series of compounds. The influence of the R_1 substituent on hemolysis can be seen, however, in the considerably lower hemolytic potency of thioridazine, in which R_1 is a methylmercapto group, as compared to NP-207 (Sandoz), which differs only in having chlorine at R_1.

Correlation of hemolytic and therapeutic potencies and partition coefficients

Statistical comparison of the therapeutic and hemolytic potencies in Table 1 gives a linear correlation coefficient (r) of 0·845, indicating a correlation that is significant at the 0·001 level.[6] The compounds that are clinically effective in the lowest dose (i.e. most potent) are also the most potent in lysing red cells, and are all located below the line in Table 1. Conversely, the compounds that require the highest clinical doses have less hemolytic activity.

All the phenothiazines used in this study were found to be easily extracted into several organic solvents from buffer (0·1 M phosphate) at pH 7·4, so that all the partition coefficients were high. This agrees with reports that phenothiazines are lipid soluble. The distributions of these compounds between organic solvents and 0·1 N HCl, however, we found to differ over a wide range, as is shown in Table 1. The data suggest that an overall inverse correlation exists between the partition coefficients ($CHCl_3$/0·1 N HCl) and the hemolytic potencies, but the linear correlation coefficient (0·56) was significant only at the 0·05–0·1 level.

Mechanism of the reaction of phenothiazines with erythrocytes

A kinetic study was made of the per cent lysis of red blood cells incubated at 37° with three different concentrations (0·5, 0·33, and 0·25 mM) of chlorpromazine. It was found that the lytic action was complete within 2 min, and the per cent of cells lysed was proportional to the amount of chlorpromazine added. Incubation for periods up to 20 min did not increase the per cent lysis above the level reached within 2 min.

To investigate why the lytic reaction was terminated so rapidly, experiments were performed with sheep erythrocyte stromata and hemoglobin (human, bovine, or sheep). Stromata bound chlorpromazine in proportion to the amount added. Hemoglobin (1–2 mg) added to the standard reaction mixture strongly inhibited the lytic reaction.

DISCUSSION

The data presented show that phenothiazines differ widely in their ability to hemolyze sheep erythrocytes. There are also considerable differences in the effective clinical doses for the phenothiazines included in this study. The test was conducted under conditions that are optimal for maintenance of the erythrocyte cell membrane, and so the effects seen are presumably concerned with disturbances of the healthy membranes of viable cells.

Table 1 shows that there is a general correlation between hemolytic activity and effective clinical dose for the phenothiazines included in this study. Such a correlation is consistent with the hypothesis of Guth and Spirtes[3] that alterations in cellular permeability are an important part of the mechanism by which phenothiazines produce tranquilization.

The structure–activity relationships in the data presented in Table 1 may also contribute to a further understanding of the mechanism of phenothiazine tranquilization. The general correlation that exists between hemolytic and clinical potencies appears to be due primarily to the effects of the side chain at position 10 (R_2), since the nature of this substituent strongly influences both activities in a similar manner. The nature of the substituent at position 2 (R_1), however, often had a less pronounced effect on hemolytic activity than on clinical effectiveness. The side chain at position 10 appears to be the part of the molecule most involved in permeability alterations, whereas the substituent at position 2 apparently influences tranquilizing effectiveness in some other way.

The results of the experiments with hemoglobin and stromata indicated that the short duration of the hemolytic reaction (less than 2 min) could be explained by the binding of phenothiazine molecules to hemoglobin and stromata from lysed cells, preventing lysis of other cells.

The degree of extractability into chloroform from acid (0·1 N HCl) appears to be influenced strongly by the nature of the R_2 substituent, since compounds with the same R_2 chain have similar partition coefficients.

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The influence of posterior pituitary hormones on drug passage into the tissue

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OUR PREVIOUS investigations[1–4] indicated that insulin controlled the accumulation of drugs in tissues both *in vitro* and *in vivo* and in this way altered the pharmacological activity of the drugs investigated.[3–4] The transport-stimulating action of insulin towards carbohydrates and amino acids may depend on the presence of disulphide bonds in the molecule.[5–11] PPH has a chemical structure which resembles insulin and Mirsky and Perisulti[12] have found that these hormones stimulated glucose uptake and utilization of fat in the epidymal pad in a similar way to insulin. This paper deals with the influence of PPH on the velocity of INH penetration and distribution and concentration in the tissues.

MATERIALS AND METHODS

Experiments were carried out with 45 rats, each weighing 150–200 g. The drugs were administered intracordially; INH—40 mg/kg, PPH—1 I.U./kg. The INH level in decapitated animals was determined in blood plasma, brain, lung, spleen and kidney at 15, 30, 45 and 60 min intervals after drug injection by the method of Deeb and Vitaglino.[14] For each point plotted 5 or 6 animals were used.

RESULTS

The experimental results are presented in Figs. 1–5. The difference in INH levels at 15, 30 and 45 min—with and without PPH injection—are significant at the 5 per cent level. The figures indicate that PPH administered together with INH increases the maximum level of INH in kidney, lung, brain, and spleen and decreases to less than half the maximum level in the blood plasma. PPH does not prolong the time taken for INH to accumulate in the tissues—the decreased level of INH in blood plasma in animals receiving PPH is probably a secondary effect due to the greater rate of INH penetration into the tissues. PPH initially increase the velocity of the drug penetration into the tissue, but subsequently increases the rate of elimination from the tissues. The effects are most pronounced in the kidney.

DISCUSSION

Previously we have found both *in vivo* and *in vitro* that insulin increased the velocity of INH penetration into lung, brain, liver but was without effect on renal tissue and plasma. Various authors[11, 12, 15, 16] have suggested that both insulin and PPH react through their -S-S groups with