International Journal of Pharmaceutics, 16 (1983) 271–283
Elsevier

Research Papers

Interaction of caffeine with phenothiazine derivatives

S.A. Khalil, L.K. EL-Khordagui and A.M. Saleh
Faculty of Pharmacy, University of Alexandria, Alexandria (Egypt)

(Received February 15th, 1983)
(Accepted March 21st, 1983)

Summary

The effect of caffeine on the partition coefficient, permeation across silastic membrane and surface activity of some phenothiazine derivatives was investigated. A decrease in the apparent iso-octane/pH 6.0 partition coefficients, permeation rate constants and surface activity of phenothiazines was indicative of a caffeine–phenothiazine interaction reducing the hydrophobicity of these drugs.

Further, such an interaction was shown to decrease the activity of phenothiazines in two simple biological systems, namely haemolysis of erythrocytes and toxicity in fish. This might have some implications for the co-administration of caffeine or caffeine-containing beverages by patients on phenothiazine therapy.

Introduction

Caffeine may be co-administered with phenothiazine drugs either as a medicinal agent (Freyhan, 1959) or more commonly in the form of caffeine-containing beverages by hospitalized mental patients (Winstead, 1976) as well as those with mild psychiatric or other disorders.

There is a controversy concerning the influence of coffee and tea consumption on the efficacy of antipsychotic drugs including phenothiazines (Kulhanek et al., 1979; Hirsch, 1979; Bowen et al., 1981). Hence, it was thought of interest to investigate the possible interaction of caffeine with phenothiazine derivatives as caffeine is known to form complexes with a variety of drugs (Higuchi and Lach, 1954; Nakano and Patel, 1970; Mattha et al., 1982).

In this study, the effect of caffeine on the physicochemical properties largely involved in the biological activity of phenothiazines is investigated. Further, the activity of phenothiazines in simple biological systems is assessed in the presence of caffeine.

0378-5173/83/$03.00 © 1983 Elsevier Science Publishers B.V.

Since structural differences may affect the interaction of the various phenothiazines, derivatives with dimethylaminoalkyl, piperidyl and piperazinyl side-chains have been selected for the study.

Materials and Methods

Equilibrium partition coefficient

The effect of caffeine [1] on the partition coefficient of promazine-HCl[2], chlorpromazine-HCl[3], triflupromazine-HCl [4], thioridazine-HCl [5], thioproperazine mesylate [6], thiethylperazine dimaleate [5], prochlorperazine-HCl [6], trifluoperazine-HCl [7] and fluphenazine-HCl [4] in iso-octane [8]–M/15 phosphate buffer, pH 6, was determined at 37°C. Twenty ml of test solutions containing 2 mM of the drug either alone or in the presence of caffeine (2–40 mM) were added to an equal volume of iso-octane in a duplicated series of glass bottles. The bottles were shaken for 10 h under exclusion of light. Samples from the aqueous layer were diluted with 0.1 N HCl and assayed spectrophotometrically at 312 nm to minimize caffeine interference.

Permeation across silastic membrane

A diffusion cell essentially similar to that described by Patel and Foss (1964) was used. The two halves of the cell were separated with a dimethylpolysiloxane (silastic) [9] membrane. One compartment of the cell was filled with 10 ml of 2 mM drug solution in M/15 phosphate buffer in the absence and presence of 2–30 mM of caffeine. The other compartment was filled with M/15 KCl–HCl buffer, pH 2.0, to maintain sink conditions in the desorbing solution. The cell was agitated in a thermostatically controlled cabinet at 37°C. At different time intervals, samples were removed from both compartments and assayed for the drug. The transport of caffeine was studied under similar conditions. Caffeine was assayed spectrophotometrically at 272 nm.

Surface tension

Surface tension measurements [10] were carried out at an ambient temperature of 18°C. Test solutions of different phenothiazine concentrations were prepared in 0.01

---

[1] Boehringer Ingelheim, F.R.G.
[2] Wyeth Laboratories, Philadelphia, PA, U.S.A.
[3] Specia, Rhône-Poulenc, France.
[4] Squibb & Sons, Inc., Princeton, NJ, U.S.A.
[5] Sandoz, A.G., Basel, Switzerland.
[6] May and Baker, Dagenham, U.K.
[7] Smith, Kline and French Laboratories, Herts, U.K.
[8] Hopkin and Williams Ltd., England.
[9] Silastic Sheeting, Dow Corning Corporation, MI, U.S.A.; 0.005 inch.
[10] Schneider Electronic Tensiometer with automatic calibration, Prôlabo, France.

M acetate buffer, pH 5.3, in the absence and presence of caffeine (2 mM). In all cases, solutions were freshly prepared and protected from light during the course of the experiment. At least 6 determinations were obtained for each drug concentration using a fresh sample for each 3 measurements.

Haemolysis study

The procedure adopted has been described in an earlier publication (El-Khordagui et al., 1980). All solutions were prepared in saline. The haemolytic effect of the appropriate concentrations of phenothiazines was determined at 37°C in the absence and presence of 2 mM caffeine using the colourimetric method of Ansel and Cadwallader (1964). The drug concentration ranges selected were those which provided full sigmoidal haemolysis curves and did not result in blood denaturation. Data presented are the means of at least 3 experiments.

Toxicity in fish

Gambusia fish (*Gambusia affinis affinis*) purchased locally were used in the study. This type of fish has been successfully used in drug absorption studies (Ghaly et al., 1975; Khalil et al., 1976). Fish weighing 200–300 mg were kept in an oxygenated aquarium for at least 7 days before experimentation. Test solutions of the phenothiazines in the absence and presence of 1 mM caffeine were freshly prepared in

TABLE 1

CHEMICAL STRUCTURES OF THE PHENOTHIAZINES UNDER STUDY

Drug	R_1	R_2
Dimethylamino derivatives		
Promazine	H	$CH_2N(CH_3)_2$
Chlorpromazine	Cl	$CH_2N(CH_3)_2$
Triflupromazine	CF_3	$CH_2N(CH_3)_2$
Piperidyl derivative		
Thioridazine	SCH_3	CH_3-N⟨ ⟩
Piperazinyl derivatives		
Thioproperazine	$SO_2N(CH_3)_2$	CH_2-N⟨ ⟩N-CH_3
Thiethylperazine	SCH_2CH_3	CH_2-N⟨ ⟩N-CH_3
Prochlorperazine	Cl	CH_2-N⟨ ⟩N-CH_3
Trifluoperazine	CF_3	CH_2-N⟨ ⟩N-CH_3
Fluphenazine	CF_3	CH_2-N⟨ ⟩N-CH_2CH_2OH

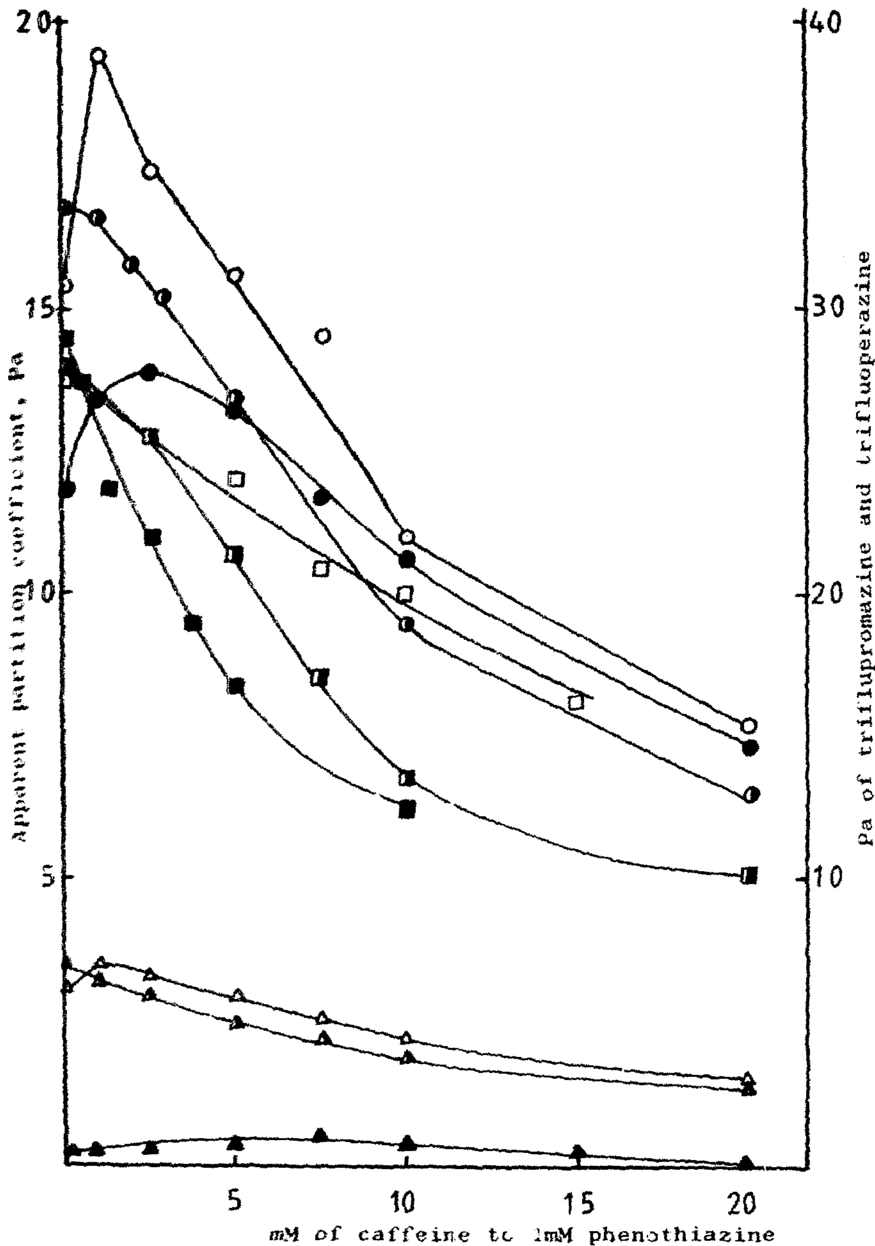

Fig. 1. Effect of caffeine on the apparent iso-octane/pH 6.0 partition coefficient of promazine (▲), chlorpromazine (■), triflupromazine (○), thioridazine (●), thioproperazine (▲), thiethylperazine (□), prochlorperazine (◨), trifluoperazine (◑), and fluphenazine (△).

distilled water and the pH was adjusted to 6.0 with dilute sodium hydroxide solution. A single fish was transferred with a plastic net to a 400 ml beaker containing 200 ml of the drug solution at 18°C and the death time was recorded. Eight to 15 fish were used at each concentration level. Drug concentration ranges selected were those which yielded a straight line when the reciprocal death time ($1/T_d$) was plotted versus concentration. The possible toxic effect of 1 mM caffeine was tested. At this concentration level, caffeine did not affect fish.

Results and Discussion

Equilibrium partition coefficient

The chemical structure of the phenothiazines used is shown in Table 1. A non-polar solvent, iso-octane, was selected as a partitioning medium to avoid the transfer of phenothiazines in the form of ion pairs with the buffer species (Murthy and Zografi, 1970). The effect of caffeine on the apparent partition coefficient of the phenothiazines under study is shown in Fig. 1. Partition coefficient generally decreased as a function of caffeine concentration. However, an initial increase could be observed in case of triflupromazine, thioridazine, fluphenazine, trifluoperazine and thioproperazine. This suggested the possible existence of these molecules in a more hydrophobic form at relatively low caffeine concentrations. At the drug concentration used in the partition study (2 mM), the above-mentioned drugs were present in the form of aggregates (Thoma and Arning, 1976a). The initial increase in their partition coefficients might imply that caffeine interfered with their aggregation in favour of the more hydrophobic monomeric form.

Nevertheless, lowering of the partition coefficients in all cases indicated a caffeine–phenothiazine interaction reducing phenothiazine hydrophobicity. The formation of a more polar caffeine–phenothiazine complex could be assumed. The presence of caffeine with phenothiazine drugs in a ratio (10: 1) corresponding approximately to the caffeine content of a cup of coffee (about 100 mg) and the normal therapeutic dose of phenothiazines resulted in a decrease in the partition coefficient of these drugs ranging from 10 to 58% (Table 2). Since many of the

TABLE 2

EFFECT OF CAFFEINE ON THE PARTITION COEFFICIENT, PERMEATION ACROSS SILASTIC MEMBRANE AND SURFACE ACTIVITY OF PHENOTHIAZINES

Drug	P_{a_1} [a]	P_{a_2} [b]	% decrease in P_a	K_1 [c]	K_2 [d]	$\pi_1 \pm$S.D. [e]	$\pi_2 \pm$S.D. [f]
Promazine	3.4	2.5	27	0.065	0.038	3.8±0.1	3.2±0.1
Chlorpromazine	14.5	6.1	58	–	–	6.4±0.1	5.6±0.5
Triflupromazine	30.9	22.5	27	–	–	11.5±0.3	11.1±0.1
Thioridazine	11.8	10.6	10	0.208	0.133	12.7±0.3	12.0±0.5
Thioproperazine	0.1	0.3 *	–	–	–	11.2±0.6	9.7±0.4
Fluphenazine	3.0	2.3	23	–	–	19.3±0.2	18.8±0.6
Thiethylperazine	13.8	8.8	36	–	–	10.3±0.1 *	10.1±0.2
Prochlorperazine	14.2	6.8	52	–	–	9.0±0.6	8.1±0.3
Trifluoperazine	33.5	19.3	42	0.299	0.234	–	–

[a] Apparent iso-octane/pH 6.0 partition coefficient in the absence of caffeine

[b] P_a in the presence of caffeine (caffeine: drug ratio is 10: 1). * At this ratio, P_{a_2} was still higher than P_{a_1} (Fig. 1).

[c] First-order rate constant, h^{-1}, for the permeation across silastic membrane.

[d] Permeation rate constant, h^{-1}, in the presence of caffeine (10: 1).

[e] Surface pressure, dynes/cm, produced by 2 mM of the phenothiazines. * π produced by 0.1 mM.

[f] Surface pressure, dynes/cm, produced by the same drug concentration in the presence of 2 mM caffeine.

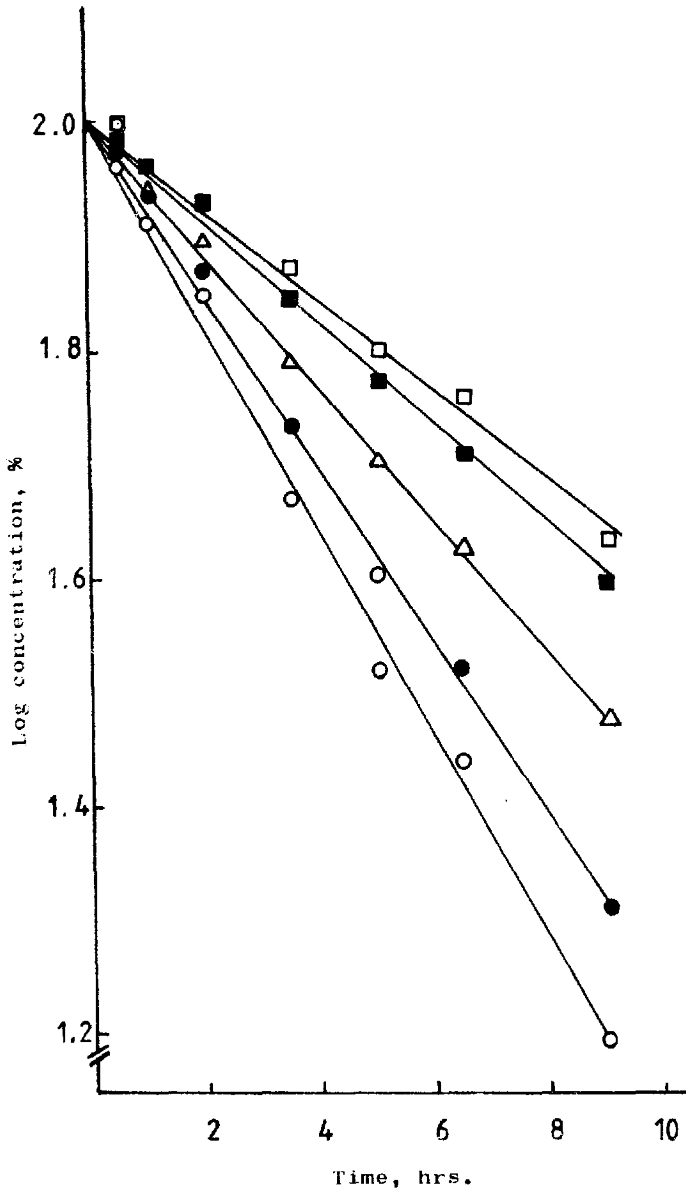

Fig. 2. Semi-log plot for the permeation of thioridazine across silastic membrane in the absence (□), and presence of 2 mM (■), 10 mM (△), 20 mM (●), and 30 mM (○) caffeine.

phenothiazine actions are dependent on their ability to accumulate in a non-polar environment (Seeman, 1972), alteration of their partition coefficient may affect their transport to such an environment. Hence, the effect of caffeine on the transport of phenothiazines across a non-polar solid membrane was studied.

Permeation across silastic membrane

Silastic membrane has been used to assess drug interactions likely to affect drug absorption (Nakano, 1971; Lovering et al., 1976). In this study, the effect of caffeine

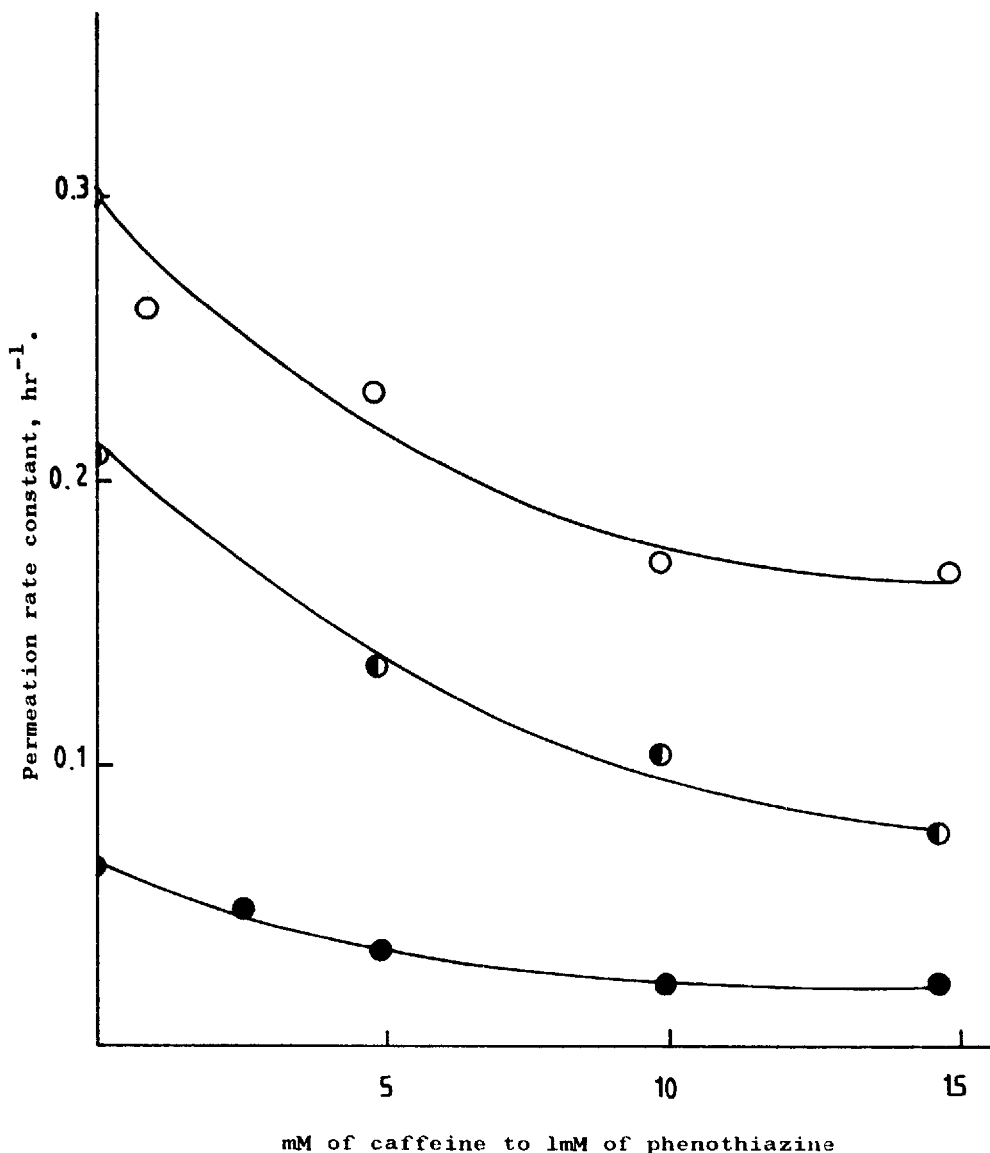

Fig. 3. Effect of caffeine on the first-order permeation rate constants of promazine (●), thioridazine (◐), and trifluoperazine (○).

on the permeation of 3 phenothiazine drugs with different side-chains, namely promazine, thioridazine and trifluoperazine, across the silastic membrane was investigated.

A semi-log plot for the disappearance of thioridazine from the diffusing compartment is shown in Fig. 2. Permeation proceeded according to first-order kinetics. The permeation rate of thioridazine decreased as a function of caffeine concentration. Similar results were obtained in the cases of promazine and trifluoperazine. Fig. 3 shows the effect of caffeine on the apparent permeation rate constants for the drugs under study. The values of the rate constants at a caffeine–drug ratio of 10 : 1 are

shown in Table 2. The transport of caffeine itself was negligible under the conditions of the experiment. Thus, the decrease in the permeation rate of these phenothiazines in the presence of caffeine supported partition coefficient data in indicating a caffeine–phenothiazine interaction in aqueous solution.

Surface activity

Surface activity is another property playing an important role in the biological activity of phenothiazines (Seeman and Bialy, 1963; Nightingale et al., 1972). The surface activity of phenothiazines may be influenced by factors affecting drug

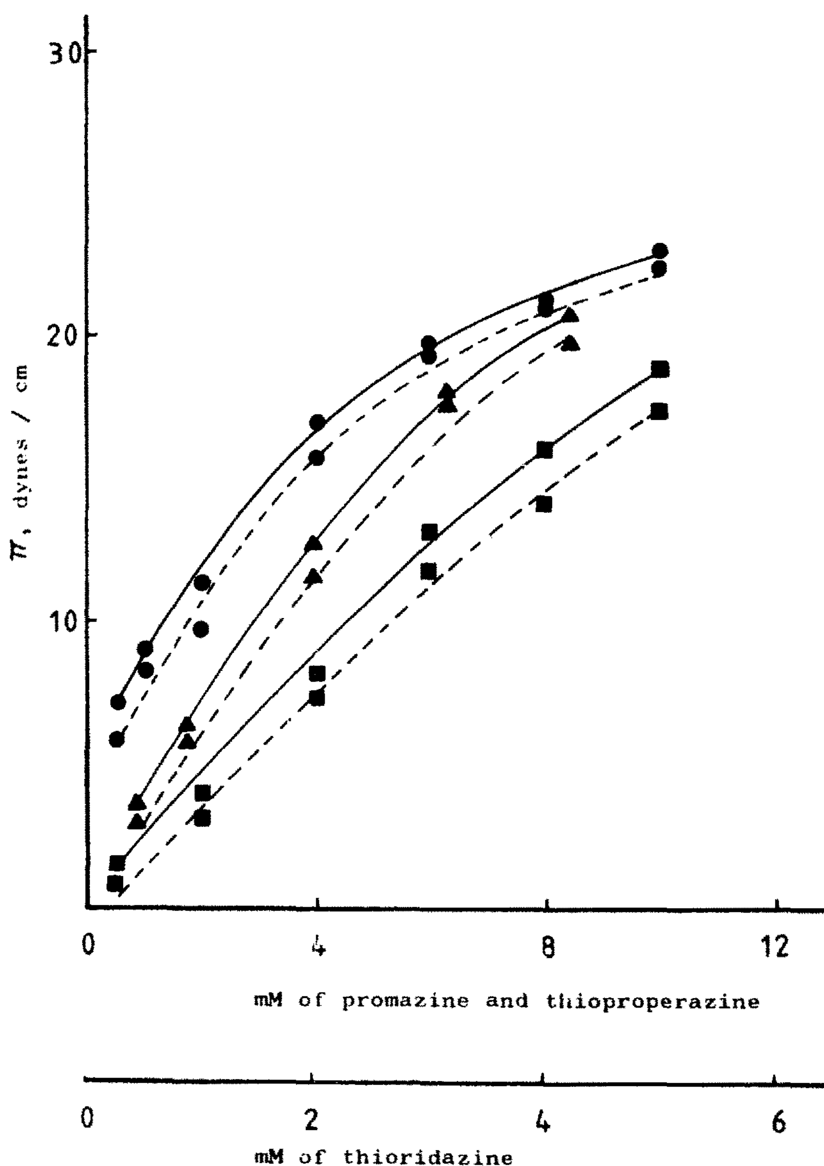

Fig. 4. Plot of surface pressure versus molar concentration of promazine (■), thioridazine (▲), and thioproperazine (●) in the absence (———) and presence (- - - - -) of 2 mM caffeine.

hydrophobicity (Patel and Zografi, 1966). In the present study, the effect of caffeine on the surface pressure, π, produced by various concentrations of the phenothiazines was investigated. The surface pressure, π, is the difference between the surface tension of the solvent and that of a given solution.

Plot of π versus molar concentration of promazine, thioridazine and thioproperazine in the absence and presence of 2 mM caffeine are shown in Fig. 4. Caffeine resulted in a decrease in π and hence the surface activity of these drugs. Similar results were obtained for the other phenothiazines under study. At each concentration level, the difference between the π values in the absence and presence of caffeine were treated statistically (Student's t-test). Although the difference was small it was significant except at relatively high drug concentrations and in the case of the highly surface active compound, thiethylperazine. The effect of caffeine (2 mM) on the surface pressure produced by an arbitrary drug concentration, 2 mM, is shown in Table 2.

The surface tension exerted by 2 mM caffeine solution was identical to that of the solvent used which excluded competition of caffeine molecules at the air–solution interface. Thus, the small decrease in the surface activity of phenothiazines in the presence of caffeine may be attributed to the formation of a more polar caffeine–phenothiazine complex with a lesser tendency to accumulate at the interface. The insignificance of the difference in the values of π at higher phenothiazine concentrations may be explained by the saturation of the surface with drug molecules so that the fraction involved in complex formation is less likely to affect the surface concentration.

The implication that the presence of caffeine might have in the biological activity

TABLE 3

EFFECT OF CAFFEINE ON THE HAEMOLYTIC ACTIVITY AND TOXICITY OF PHENOTHIAZINES IN FISH

Drug	$C_{50\%}$ [a]	$C_{50\%}$ in presence of 2 mM caffeine	Slope [b] $\times 10^3$	Slope $\times 10^3$ in presence of 1 mM caffeine	T_d [c]	T_d in presence of 1 mM caffeine
Promazine	2.08	2.20	28	24	13.0	15.9
Chlorpromazine	0.71	0.76	32	28	12.1	12.7
Triflupromazine	0.57	0.62	44	38	11.4	12.2
Thioridazine	0.20	0.26	48	40	9.5	10.2
Thioproperazine	0.20	0.22	140	122	9.6	9.9
Prochlorperazine	0.17	0.18	82	59	13.5	15.2
Trifluoperazine	–	–	64	51	10.8	13.2
Fluphenazine	0.14	0.15	58	54	10.2	14.5
Thiethylperazine	0.08	0.09	530	455	16.8 *	17.2

[a] Concentration (mM of phenothiazines inducing 50% haemolysis) values obtained from the haemolysis curves.

[b] Regression slopes of reciprocal death time versus concentration plots.

[c] Time (min) of death of fish induced by 0.5 mM of the various phenothiazines. * Time of death induced by 0.1 mM.

of phenothiazines was then tested using two simple biological systems, namely haemolysis of erythrocytes and toxicity in fish.

Haemolytic activity

The erythrocyte membrane is an intact readily accessible biological membrane known to respond to phenothiazines (Seeman, 1972). The haemolytic activity of the

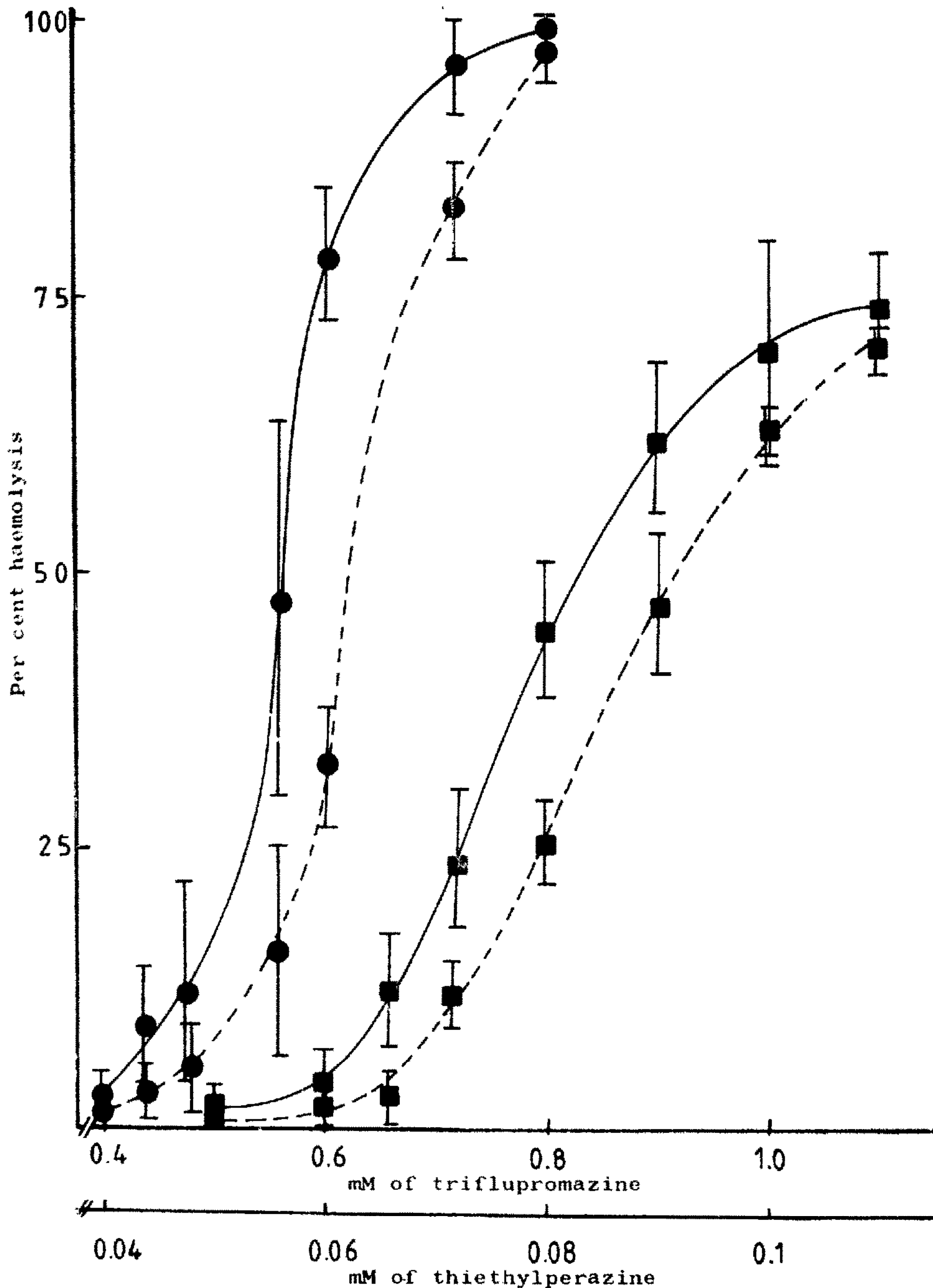

Fig. 5. Haemolytic activity of triflupromazine (●), and thiethylperazine (■) in the absence (———) and presence (- - - - - -) of 2 mM caffeine. Points represent the mean ± S.D.

phenothiazines under study was tested in the presence of 2 mM caffeine. The haemolytic effect of up to 4 mM caffeine in saline was negligible. In all cases, haemolysis curves were obtained by plotting the percent haemolysis versus molar concentration of the drug in the absence and presence of caffeine. Fig. 5 shows the haemolysis curves for triflupromazine and thiethylperazine. All the phenothiazines studied exhibited a similar pattern, which indicated a decrease in the haemolytic activity of these drugs in the presence of caffeine. Such a decrease was statistically significant at intermediate phenothiazine concentrations corresponding to the central portions of the sigmoidal haemolysis curves. Drug concentrations inducing 50% haemolysis in the absence and presence of caffeine are presented in Table 3.

The apparent stabilization of erythrocytes against the haemolytic effect of phenothiazines may be attributed to an interaction reducing the tendency of these drugs to accumulate at the biological membrane. A direct stabilizing effect of caffeine on the membrane through binding with its components may also be taken into consideration. However, as the affinity of caffeine for binding with protein is very

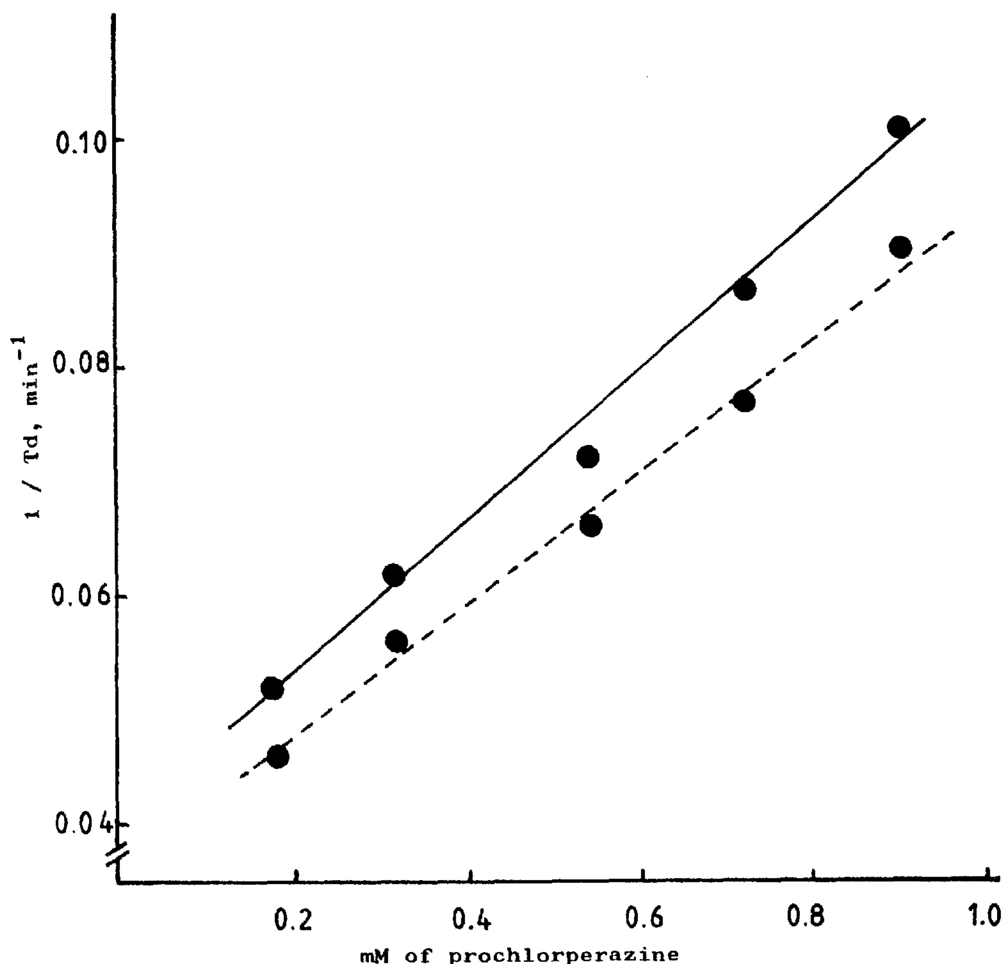

Fig. 6. Reciprocal death time versus molar concentration of prochlorperazine in the absence (———) and presence (- - - - - -) of 1 mM caffeine.

low (Eichman et al., 1962) compared to that of phenothiazines (Thoma and Arning, 1976b), the first explanation seems reasonable.

Toxicity in fish

The caffeine–phenothiazine interaction was further assessed using a simple living system, fish. Sensitivity of fish to changes in the physicochemical properties of drug molecules made it a potentially useful tool for the study of drug interactions (Ghaly et al., 1975; Frömming and Ghaly, 1981). Toxicity plots for the phenothiazines under study were obtained by plotting the reciprocal of death time ($1/T_d$) versus drug concentration in the absence and presence of 1 mM caffeine. Fish survived in 1 mM caffeine solution for more than 48 h. Fig. 6 shows the toxicity plot for prochlorperazine. Caffeine decreased the drug-induced toxicity as indicated by the lower slope of the line and the longer survival time. Similar results were obtained in all cases. Values of the slopes of the regression lines for the phenothiazines studied and the time of death induced by an arbitrary drug concentration, 0.5 mM, are presented in Table 3. The effect of caffeine on death time was statistically significant except in case of thioproperazine and thiethylperazine.

The decreased toxicity of phenothiazines in the presence of caffeine may be attributed to a decrease in the bioavailability of these drugs to fish as a result of interaction with caffeine. However, contribution of the pharmacological activity of caffeine could not be ignored.

Thus, apart from the reported physical incompatibility between phenothiazines and tea and coffee (Kulhanek et al., 1979), caffeine reduces the hydrophobicity of this group of drugs. This is indicated by the lower partition coefficient, transport rate across silastic membrane and surface activity of the phenothiazines in the presence of caffeine.

It could be concluded that the lower activity of phenothiazines in the biological systems employed in this study and the possible weakening of the effect of antipsychotic medication due to increased coffee consumption (Kulhanek, 1979) may not be totally discussed in the context of pharmacological activity.

Nevertheless, limitations on the intake of caffeine or caffeine-containing beverages by patients on phenothiazine therapy could not be justified unless the clinical implications of the caffeine–phenothiazine interaction are assessed. Although such limitations have not been justified in a recent study, (Bowen et al., 1981), the results might be misleading in view of the wide variation in blood levels reported. Hence, further studies are indispensable.

References

Ansel, H.C. and Cadwallader, D.E., Haemolysis of erythrocytes by antibacterial preservatives. J. Pharm. Sci., 53 (1964) 169–172.

Bowen, S., Taylor, A.K. and Gibb, I.A.McI., Effect of coffee and tea on blood levels and efficacy of antipsychotic drugs. Lancet, i (1981) 1217–1218.

Eichman, M.L., Guttman, D.E., van Winkle, Q. and Guth, E.P., Interactions of xanthine molecules with bovine serum albumin. J. Pharm. Sci., 51 (1962) 66–71.

El-Khordagui, L.K., Saleh, A.M. and Khalil, S.A., Diazepam sodium salicylate solution: dilution with intravenous fluids, in vitro haemolytic activity and protein binding. Int. J. Pharm., 7 (1980) 111-118.

Freyhan, F.A., Therapeutic implications of differential effects of new phenothiazine compounds. Am. J. Psychiat., 115 (1959) 577-585.

Frömming, K.-H. and Ghaly, G.M., Influence of pluronic F68 on the absorption of benzocaine and lidocaine by fish. Arch. Pharm., 11 (1981) 926-932.

Ghaly, G.M., Khalil, S.A., Moustafa, M.A., Gouda, M.W. and Motawi, M.M., Effect of complexation with some xanthines on the availability of chlorpromazine. Can. J. Pharm. Sci., 10 (1975) 79-82.

Higuchi, T. and Lach, L. Investigation of some complexes formed in solution by caffeine. J. Am. Ass., Sci. Edn., 43 (1954) 349-354.

Hirsch, S.R., Precipitation of antipsychotic drugs in interaction with coffee or tea. Lancet, ii (1979) 1130-1131.

Khalil, S.A., Abdallah, O.Y. and Moustafa, M.A., Absorption of some barbiturates by Gambusia fish and its correlation to solubility parameter. Can. J. Pharm. Sci., 11 (1976) 26-30.

Kulhanek, F., Linde, O.K. and Meisenberg, G., Precipitation of antipsychotic drugs in interaction with coffee or tea. Lancet, ii (1979) 1130.

Lovering, E.G., Mainville, C.A. and Rowe, M.L., Drug permeation through membranes V: Interaction of diazepam with common excipients. J. Pharm. Sci., 65 (1976) 207-210.

Mattha, A.G., Ammar, H.O., Abdel-Samie, M., EL-Nahhas, S.A. and Kassem, M.A., Complex formation between antihistaminic drugs and caffeine. Pharm. Acta Helv., 57 (1982) 268-272.

Murthy, K.S. and Zografi, G., Oil-water partitioning of chlorpromazine and other phenothiazine derivatives using dodecane and *n*-octanol. J. Pharm. Sci., 59 (1970) 1281-1285.

Nakano, M. and Patel, N.K., Effect of molecular interaction on permeation of organic molecules through dimethylpolysiloxane membrane. J. Pharm. Sci., 59 (1970) 77-81.

Nakano, M., Effect of interaction with surfactants, adsorbents and other substances on the permeation of chlorpromazine through a dimethylpolysiloxane membrane. J. Pharm. Sci., 60 (1971) 571-575.

Nightingale, C.H., Tse, M. and Stupack, E.I., Structure-toxicity relationships of substituted phenothiazines. J. Pharm. Sci., 61 (1972) 1498-1499.

Patel, N.K. and Foss, N.E., Effect of temperature on the binding of parabens and phenols by polysorbate 80 and polyethylene glycol 4000. J. Pharm. Sci., 53 (1964) 94-97.

Patel, R.M. and Zografi, G., Factors influencing the surface activity of chlorpromazine at the air-solution interface. Effect of inorganic and organic electrolytes. J. Pharm. Sci., 55 (1966) 1345-1349.

Seeman, P. and Bialy, H.S., The surface activity of tranquilizers. Biochem. Pharmacol., 12 (1963) 1181-1191.

Seeman, P., The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev., 24 (1972) 583-655.

Thoma, K. and Arning, M., Colloidal, partition and binding properties of phenothiazine derivatives I. Relationships between chemical structure and molecular association. Arch. Pharm., 309 (1976a) 837-850.

Thoma, K. and Arning, M., Colloidal, partition and binding properties of phenothiazine derivatives V. Relationships between albumin binding and physical properties. Arch. Pharm., 309 (1976b) 945-959.

Winstead, D.K., Coffee consumption among psychiatric inpatients. Am. J. Psychiat., 133 (1976) 1447-1450.