BBA 43270

Effect of phenothiazines and their sulfoxides on lipoamide dehydrogenase

Chlorpromazine and other phenothiazine derivatives are known to uncouple oxidative phosphorylation and inhibit respiration. Both flavin- and NADH-linked enzyme systems have been implicated as sites for phenothiazine inhibition. (For extensive reviews see refs. 1 and 2.) D-Amino-acid oxidase (D-amino-acid:O_2 oxidoreductase (deaminating), EC 1.4.3.3) has been studied as a model flavoprotein[1,3]. It was found that chlorpromazine not only complexes with FAD but also competes with apoenzyme for FAD.

We examined the effect of phenothiazine derivatives on another flavoprotein, lipoamide dehydrogenase (NADH:lipoamide oxidoreductase, EC 1.6.4.3). We have previously shown that purified pig brain and heart lipoamide dehydrogenase are identical and are competitively inhibited by folic acid and unconjugated pteridines[4].

Pig heart lipoamide dehydrogenase (Sigma) was assayed for lipoamide dehydrogenase, transhydrogenase and diaphorase activity in the presence of phenothiazine as previously described[4]. Chlorpromazine, trifluorperazine, and chlorpromazine sulfoxide were donated by Smith, Kline and French. Trifluorperazine sulfoxide was prepared by reaction of trifluorperazine with H_2O_2. The recrystallized trifluorperazine sulfoxide was identified by absorption at 303 and 273 nm.

In contrast to D-amino-acid oxidase, lipoamide dehydrogenase was not inhibited by chlorpromazine or trifluorperazine at concentrations up to $1 \cdot 10^{-3}$ M. Similarly, preincubation of enzyme with phenothiazine at 0° for 30 min had no effect on enzyme activity. However, chlorpromazine sulfoxide and trifluorperazine sulfoxide, which do not inhibit D-amino-acid oxidase, were competitive inhibitors of lipoamide dehydrogenase with K_i's of $3.3 \cdot 10^{-2}$ mM and $16 \cdot 10^{-2}$ mM, respectively, as determined by DIXON[5] plots (Fig. 1).

Besides NADH lipoamide oxidoreduction, lipoamide dehydrogenase also catalyzes transhydrogenation (*e.g.* thionicotinamide NAD^+–NADH) and a diaphorase reaction (dichlorophenolindophenol–NADH). These three functions of the enzyme

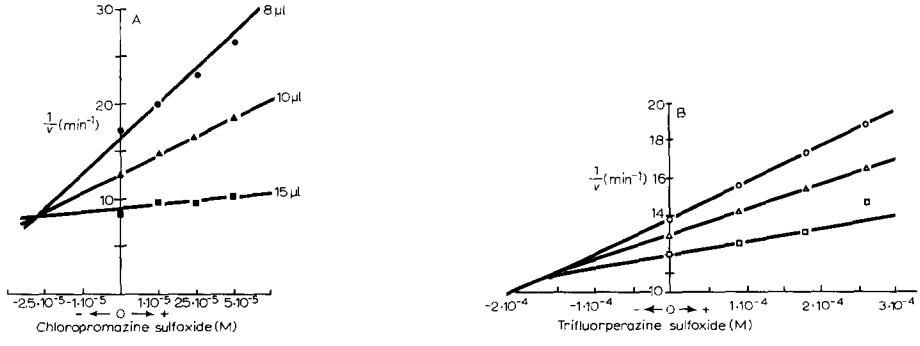

Fig. 1. Determination of K_i for (A) chlorpromazine sulfoxide and (B) trifluorperazine sulfoxide with pig heart lipoamide dehydrogenase. Reaction medium contained 3 ml 0.05 M potassium phosphate (pH 6.0), 1.13 mM EDTA, 2 mg bovine serum albumin, 0.1 mM NAD^+, 0.1 mM NADH, 1.3 µg lipoamide dehydrogenase, and DL-lipoamide and inhibitor as indicated: (A) lipoamide, 0.10 mM (●), 0.14 mM (▲), 0.20 mM (■); and (B) 0.33 mM (○), 0.45 mM (△), 0.64 mM (□).

respond differently to inhibitors[4,6] and, indeed, phenothiozine sulfoxides did not inhibit either transhydrogenase or diaphorase reactions of pure lipoamide dehydrogenase.

The inhibition of lipoamide dehydrogenase by the phenothiazine sulfoxides is not due to the sulfoxide group *per se* since neither dimethylsulfoxide nor methionine sulfoxide at $1 \cdot 10^{-3}$ M had any effect, nor did methionine sulfoximine. Charge transfer interactions are often invoked to explain chlorpromazine inhibition of enzymes[1]. However, oxidation to the sulfoxide should greatly diminish the molecule's donor properties[7]. When chlorpromazine and riboflavin at $1 \cdot 10^{-3}$ M are frozen, the color changes from yellow to brownish-green and this has been ascribed to a charge-transfer complex between the two compounds[8]. In contrast, we found no observable color change on freezing a similar equimolar solution of chlorpromazine sulfoxide and riboflavin.

Conclusions as to a site of inhibition from kinetic data must be made with caution. However, some conclusions can be drawn from these experiments. Phenothiazine sulfoxides do not compete for an active site or interact with NAD^+–NADH. Such action should cause inhibition of the transhydrogenase activity as well. Reaction with the sulfhydryl groups of the enzyme necessary for lipoamide reductase activity is also unlikely. Oxidation of sulfhydryl groups by cupric ion or reaction of these groups with mercurial results in an increased diaphorase activity concommitant with loss in lipoamide dehydrogenase activity[6]. This result was not found in the case of phenothiazine sulfoxides. Additionally, a chemical reaction by phenothiazine sulfoxides with an active site (such as oxidation of sulfhydryl) is unlikely since dimethyl sulfoxide and methionine sulfoxide were not inhibitory. It is more likely the competitive inhibition of lipoamide dehydrogenase by phenothiazine sulfoxides could best be ascribed to the competition of these molecules with lipoamide for the reductive site on the enzyme.

It is possible that the inhibition of lipoamide dehydrogenase by phenothiazine sulfoxides may contribute to the physiological effects of the parent drug because chlorpromazine sulfoxide comprises about 10% of the *in vivo* metabolites of chlorpromazine.

This work was supported by a grant from the State of Iowa Mental Health Fund. The technical assistance of Mr. Robert Fetherolf is gratefully acknowledged.

Division of Neurobiochemistry, SARA A. MILLARD
Department of Psychiatry, College of Medicine,
University of Iowa,
Iowa City, Iowa 52240 (U.S.A.)

1 S. GABAY AND S. R. HARRIS, in J. L. RABINOWITZ AND R. M. MYERSON, *Topics in Medicinal Chemistry*, Vol. 3, John Wiley, New York, 1970, p. 57.
2 J. A. BAIN AND S. E. MAYER, *Ann. Rev. Pharmacol.*, 2 (1962) 37.
3 K. YAGI, T. OZAWA AND T. NAGATSU, *Biochim. Biophys. Acta*, 43 (1960) 310.
4 S. A. MILLARD, A. KUBOSE AND E. M. GÁL, *J. Biol. Chem.*, 244 (1969) 2511.
5 M. DIXON, *Biochem. J.*, 55 (1953) 170.
6 L. CASOLA, P. E. BRUMBY AND V. MASSEY, *J. Biol. Chem.*, 241 (1966) 4977.
7 B. PULLMAN AND A. PULLMAN, *Biochim. Biophys. Acta*, 35 (1959) 535.
8 G. KARREMAN, I. ISENBERG AND A. SZENT-GYORGYI, *Science*, 30 (1959) 1191.

Received March 17th, 1970