Biochemical Pharmacology, 1963, Vol. 12, pp. 19–24. Pergamon Press Ltd., Printed in Great Britain.

DIFFERENTIAL EFFECTS OF PHENOTHIAZINES ON HEXOSE PHOSPHATE DEHYDROGENASES

MICHAEL J. CARVER

Nebraska Psychiatric Institute and Department of Biochemistry,
University of Nebraska College of Medicine, Omaha, Nebraska, U.S.A.

(*Received* 18 *June* 1962; *accepted* 21 *August* 1962)

Abstract—Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from beef adrenal were inhibited by a variety of substituted phenothiazines. With similar enzymes isolated from rat brain tissue only glucose-6-phosphate dehydrogenase was affected. Kinetic studies with the brain enzyme gave no evidence of competitive inhibition. Results with imipramine, including kinetic studies, were in most instances similar to those obtained with the phenothiazines.

RECENT studies have indicated that phenothiazine derivatives are inhibitors of G-6PD* from erythocytes.[1] It has been further demonstrated that thioproperazine will inhibit both G-6PD and 6-PGD from bovine adrenal cortex and that the inhibition observed is noncompetitive with the first enzyme and competitive with the latter. With either enzyme, however, preincubation of the phenothiazine was required for the inhibition.[2]

As an extension of this work, a comparison was made of the inhibitory effects of various phenothiazine derivatives on G-6PD and 6-PGD from beef adrenal cortex and rat brain. In addition, some tentative information was obtained on structure-activity relations with these phenothiazine derivatives.

MATERIALS AND METHODS

The enzymes from beef adrenal cortex and rat brain were prepared as previously described.[3] Briefly, a 20% homogenate of the tissue in distilled water was centrifuged at 4° for 30 min at 20,000 × *g*. The supernatant solution was dialyzed in the cold overnight and then equal volumes of the supernatant solution and saturated ammonium sulfate solution were mixed. The precipitate obtained was collected by centrifugation and contained most of the G-6PD of the original homogenate. Repetition of the ammonium sulfate precipitation resulted in a preparation essentially free of 6-PGD activity. The supernatant solution remaining after the first ammonium sulfate precipitation contained the 6-PGD activity and was used as such.

G-6PD assays were based on the method of Kornberg and Horecker,[4] 6-PGD assays on the method of Horecker and Smyrniotis.[5] One unit of activity was defined as the amount of enzyme causing the reduction of 0·01 μmole of TPN/min at 25°.

Although a large number of phenothiazine derivatives is available, the particular ones used in this study were chosen on the basis of their solubility in glycylglycine

* Abbreviations: G-6PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconate dehydrogenase; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; TPN, triphosphopyridine nucleotide; G-6-P, glucose-6-phosphate; 6-PG, 6-phosphogluconate.

buffer, pH 7·4. Each compound was screened for its solubility at pH 7·4 and at the concentration used in the reaction cuvet. With such criteria the number of satisfactory compounds was not extensive (Fig. 1). Since there is some question about the stability of phenothiazines they were prepared fresh daily.

RESULTS

The inhibitory effects of the compounds studied are presented in Table 1. When the phenothiazine was preincubated for 30 min in the presence of either enzyme from the

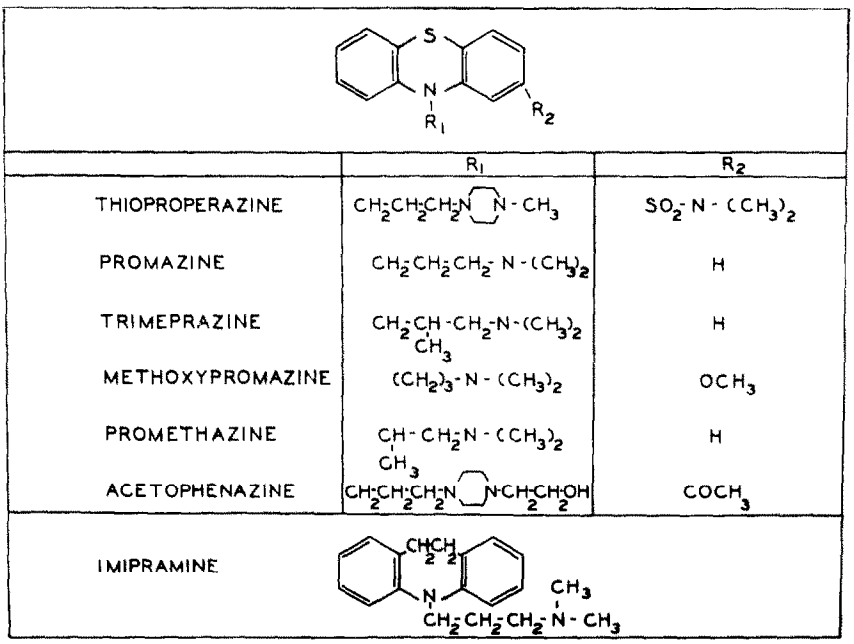

FIG. 1. Compounds used in this study.

TABLE 1. INHIBITORY EFFECTS OF COMPOUNDS ON DEHYDROGENASES*

Compounds	Beef adrenal cortex				Rat brain			
	G-6PD		6-PGD		G-6PD		6-PGD	
	a	b	a	b	a	b	a	b
Thioproperazine	62·3	0	54·2	33·3	46·2	0	0	0
Promazine	45·5	0	39·8	0	44·5	0	0	0
Acetophenazine	44·5	9·2	40·0	0	54·8	33·4	0	0
Methoxypromazine	43·3	0	51·7	0	35·5	0	0	0
Trimeprazine	33·1	0	35·6	0	40·0	0	0	0
Promethazine	26·5	0	30·7	0	27·1	0	0	0
Imipramine	25·5	0	0	0	20·9	0	0	0

* Values are % inhibition of enzyme activity expressed in terms of the rate of the reaction in the presence of phenothiazine compared with that in its absence; (a): preincubation of phenothiazine with complete system minus TPN and G-6-P for 30 min; (b): preincubation of phenothiazine with complete system minus G-6-P for 30 min.

System. All cuvets contained 250 μmole glycylglycine buffer, pH 7·4; 100 μmole $MgCl_2$; 0·23 μmole TPN; and 5 μmole G-6-P or 6-PG; inhibitor, 1·5 μmole in total volume of 3·0 ml. Added to various experiments: adrenal G-6PD, 1 mg protein (4 to 6 units of activity); adrenal 6-PGD, 1 mg protein (5 to 8 units of activity); brain G-6PD, 1 mg protein (3·8 units of activity); brain 6-PGD, 1 mg protein (2·0 units of activity).

beef adrenal cortex, significant inhibition of the system occurred. For example, with G-6PD the inhibition varied from 26·5% with promazine to 62·3% with thioproperazine. On the other hand, incubation of the phenothiazines and TPN with the enzyme prior to the addition of the substrate completely abolished the inhibitory effects, acetophenazine being the only exception.

G-6PD from rat brain behaved like the beef adrenal enzyme, in that inhibition from 27·1 to 54·8 per cent was noted. Moreover, the preincubation of the phenothiazines and TPN abolished the inhibitory effect, with the exception of acetophenazine. Rat brain 6-PGD, however, was entirely unaffected by the concentrations used in this study.

Since it had been previously noted that thioproperazine was a noncompetitive inhibitor of G-6PD and a competitive inhibitor of 6-PGD, further studies with this phenothiazine were carried out on the rat brain G-6PD to determine the nature of the inhibition. Activity was measured in the presence of a constant amount of inhibitor (5×10^{-4} M), first with an excess of TPN and limiting concentration of G-6-P and second, with excess of G-6-P and limiting concentrations of TPN (Fig. 2). No evidence of competitive inhibition was noted.

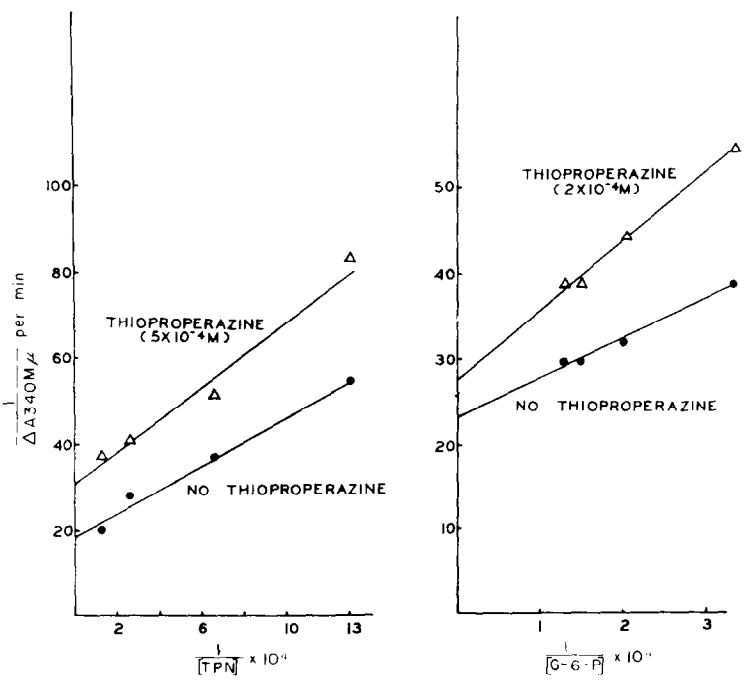

FIG. 2. Relation between 1/V and 1/S [(1/Δ absorbancy at 340 mμ/min) and (1/(G-6-P) $\times$ 10^4 *or* 1/TPN $\times$ 10^4)] in the presence and absence of a constant amount of thioproperazine. On the left the concentration of TPN was varied, on the right the concentration of G-6-P was varied. The system included: enzyme, 1 mg protein (specific activity 3·8 units/mg protein); 250 μmole glycylglycine buffer, pH 7·4; 100 μmole $MgCl_2$; and either 0·23 μmole TPN or 5 μmole G-6-P in a total volume of 3·0 ml. Activity in the presence of thioproperazine was measured after preincubation of the drug for 30 min with the complete system minus either TPN or G-6-P.

Studies with imipramine

Since in many instances the biochemical effects of imipramine and phenothiazines on mitochondria and tissue metabolism are similar, the effects of imipramine were evaluated. Examination of the data in Table 1 suggests that imipramine inhibits the G-6PD as do the phenothiazines. Moreover, Lineweaver-Burk plots[6] (Fig. 3) indicate that this compound is not a competitive inhibitor of rat brain G-6PD. No effect was observed on 6-PGD from either beef adrenal cortex or rat brain.

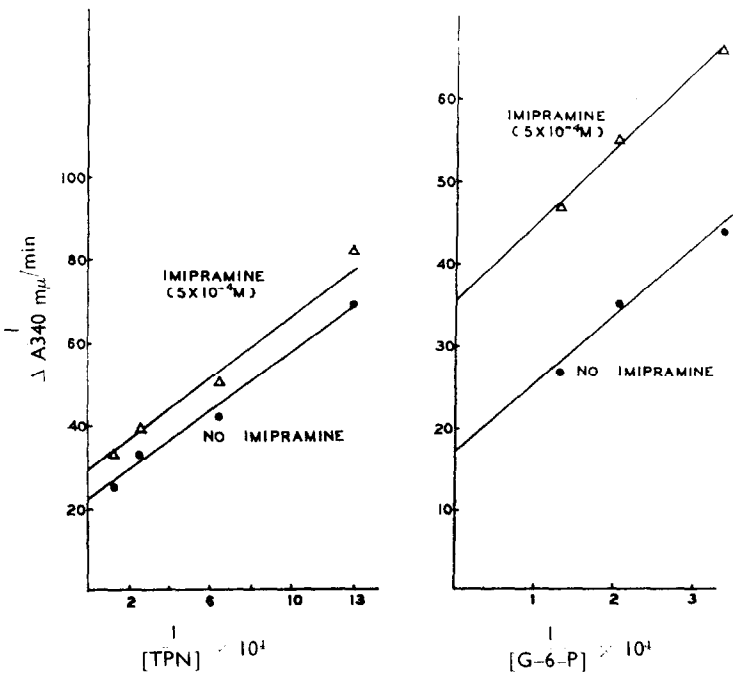

FIG. 3. Relation between 1/V and 1/S in the presence and absence of imipramine. Conditions as indicated in Fig. 2.

Structure-activity relations

Comparison of the results obtained in this study indicates that each of the phenothiazines inhibited the enzymes from beef adrenal and the G-6PD from rat brain. None was effective on 6-PGD from rat brain. Wherever inhibition was noted, promethazine was the least effective. Imipramine, on the other hand, exerted no effect on 6-PGD from adrenal and in those systems where inhibition did occur, it was less effective than any of the phenothiazines.

It appears that the sulfur in the ring is not necessary for an inhibitory effect on G-6PD, since imipramine is active. The presence of sulfur, however, contributes to inhibition (compare, for example, promazine and imipramine). It is also of interest that the isomers, promethazine and promazine, differ significantly in their inhibitory effect. The data are not sufficient to explain the role of R_2, although it tentatively appears that in most instances substitutions here favor inhibition.

DISCUSSION

The data presented above are consistent with the concept that phenothiazines are inhibitors of both G-6PD and 6-PGD from beef adrenal cortex and of G-6PD from rat brain tissue. Although the degree of inhibition varies to some extent with the phenothiazine under study, kinetic studies with thioproperazine indicate that the inhibition is similar with G-6PD from bovine adrenal and rat brain.

On the other hand, the basic nature of the inhibition of 6-PGD is different. With the adrenal enzyme, competitive inhibition was noted with thioproperazine; however, no inhibition of the rat brain enzyme could be obtained with a wide variety of phenothiazines. No immediate explanation is apparent from the data, although it is known that the two shunt enzymes act differently, depending upon the experimental conditions.[7, 8] It has also been observed[9] that pregnenolone and dehydroisoandrosterone inhibit G-6PD from several sources but not from spinach or yeast and not 6-PGD from several tissues (adrenal, liver, adipose). Alternative suggestions for the lack of effect on 6-PGD might be sought in the presence of natural inhibitors of phenothiazines in the enzyme preparation or conceivably TPN could be tightly bound to the apoenzyme, thus preventing phenothiazine-enzyme interaction. In any event, it has been possible to demonstrate a differential effect by phenothiazines on these two closely related enzymes of the hexose monophosphate pathways.

Imipramine, although not a phenothiazine, resembles the phenothiazines to some extent. It inhibits respiratory activity of rat brain, the uptake and incorporation of glycine-1-^{14}C[10] and oxidative phosphorylation in rat liver and brain mitochondria. These effects can also be demonstrated with chlorpromazine.[11, 12] The present data are also consistent with the idea that imipramine and the phenothiazines have much in common biochemically. Imipramine inhibition of G-6PD from bovine adrenal and rat brain was comparable to promethazine inhibition. Furthermore, the kinetic studies using this compound suggest a similar inhibition of rat brain G-6PD. An inconsistency was demonstrated, however, in that imipramine exerted no inhibitory effect on 6-PGD from bovine adrenal.

In general, the results of this investigation are similar to those previously obtained in the study of D-amino acid oxidase [13, 14] and the interaction of FAD, chlorpromazine, and apoenzyme and also in the study of the interaction of FMN, chlorpromazine, and the old yellow enzyme.[15] In these investigations, as well as in the present one, interaction of a phenothiazine with coenzyme for the apoenzyme was demonstrated.

Any attempt to generalize on structure-inhibitory relations reported here has severe limitations. The difference in inhibitory effect between many of the phenothiazines is small. Moreover, the degree of purity of the enzyme preparation is such that nonenzymic protein could decrease the inhibition by binding the compound under study. However, attempts at finding relationships between enzymic effects and structure should be made since they may lead to a better understanding of drug action.

It is exceedingly difficult to establish the primary pharmacological action of the phenothiazines since they affect a wide variety of biochemical processes.[16–19] Further, most studies have been carried out on *in-vitro* systems and cannot be taken as evidence of a similar *in-vivo* response.[20] It is worthy of note, however, that the enzymes studied here are components of a key system for the generation of TPNH.

The importance of this coenzyme in biosynthetic mechanisms is well known;[21] however, the low concentration of TPN[22, 23] in brain suggests that the activity of the

hexose monophosphate shunt is minimal[24] in this tissue. If, on the other hand, TPN is added to brain mince in physiological concentrations, activity of the shunt is appreciably increased.[25] The significance, therefore, of present investigations must await further evaluation of the shunt in brain.

Acknowledgments—The author gratefully acknowledges the valuable assistance of Miss Nadine Roesky in this work. Appreciation is also expressed to Smith, Kline & French, Wyeth, Geigy, and Lederle for the compounds used. The study was supported by the Nebraska Fund for Psychiatric Research.

REFERENCES

1. M. J. CARVER, J. D. MARKS and N. ROESKY, *Experientia, Basel* **17**, 315 (1961).
2. J. D. MARKS, N. ROESKY and M. J. CARVER, *Arch. Biochem.* **95**, 192 (1961).
3. T. L. KELLY, J. D. NIELSON, R. B. JOHNSON and C. S. VESTLING, *J. biol. Chem.* **212**, 545 (1955).
4. A. KORNBERG and B. L. HORECKER, *Methods in Enzymology* p. 323, vol. I, Academic Press, New York (1955).
5. B. L. HORECKER and P. SMYRNIOTIS. *Ibid.* p. 323.
6. H. LINEWEAVER and D. BURK, *J. Amer. chem. Soc.* **56**, 658 (1934).
7. W. M. FITCH, R. HILL and I. L. CHAIKOFF, *J. biol. Chem.* **234**, 1048 (1959).
8. G. E. GLOCK and P. MCLEAN, *Biochem. J.* **61**, 390 (1955).
9. P. A. MARKS and J. BANKS, *Proc. nat. Acad. Sci., Wash.* **46**, 447 (1960).
10. P. N. ABADOM, K. AHMED and P. G. SCHOLEFIELD, *Canad. J. Biochem.* **39**, 551 (1961).
11. M. J. R. DAWKINS, J. D. JUDAH and K. D. REES, *Biochem. J.* **72**, 204 (1959).
12. *Ibid.* **73**, 16 (1959).
13. K. YAGI, T. OZAWA and T. NAGATSU, *Biochim. biophys. Acta* **43**, 310 (1960).
14. A. LASSLO and A. L. MEYER, *Nature, Lond.* **184**, 1922 (1959).
15. S. KISTNER, *Acta chem. scand.* **14**, 1389 (1960).
16. J. BERNSOHN, I. NAMAJUSKA and B. BOSHES, *J. Neurochem.* **1**, 145 (1955).
17. L. G. ABOOD, *Proc. Soc. exp. Biol., N.Y.* **88**, 688 (1955).
18. R. G. GRENELL, J. MENDELSON and W. D. MCELROY, *Arch. Neurol. Psychiat., Chic.* **73**, 347 (1955).
19. E. W. HELPER, M. J. CARVER, H. P. JACOBI and J. A. SMITH, *Arch. Biochem.* **76**, 354 (1958).
20. A. D. WELCH and E. BUEDING, *Currents in Biochemical Research*, Interscience, New York (1946).
21. B. L. HORECKER and H. H. HIATT, *New Engl. J. Med.* **258**, 225 (1958).
22. N. KAPLAN, *Neurochemistry of Nucleotides and Amino Acids*. Wiley, New York (1960).
23. G. E. GLOCK and P. MCLEAN, *Biochem. J.* **61**, 388 (1955).
24. D. B. TOWER, *J. Neurochem.* **3**, 185 (1958).
25. S. S. HOTTA, *J. Neurochem.* **9**, 43 (1962).