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IN VIVO AND *IN VITRO* STUDIES ON THE EFFECTS OF SOME PHENOTHIAZINES AND SULPIRIDE ON KYNURENINE METABOLISM

M. H. MOSTAFA, S. M. EL-SEWEDY, E. A. EL-BASSIOUNI* and G. A. ABDEL-TAWAB
Medical Research Institute and University of Alexandria Research Center (U.N.A.R.C.), Alexandria University, Alexandria, Egypt

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Abstract—The effect of 5 consecutive daily i.p. doses of CPZ (5 mg/kg), PZ (10 mg/kg) and PMZ (10 mg/kg) on the activity of kynurenine hydrolase and kynurenine aminotransferase in mouse liver was studied. All three phenothiazines effected an increase in the activity of kynurenine hydrolase per unit weight of liver with CPZ showing the highest activation followed by PZ and PMZ. On the other hand kynurenine aminotransferase was more moderately inhibited by the above treatment. *In vitro* studies showed that the phenothiazines tested and the pharmacologically inactive CPZO, the major metabolite of CPZ, in concentrations ranging from 3×10^{-9} to 3×10^{-4} M had an activating effect on kynurenine hydrolase. Increases in activity were obtained up to concentrations of 3×10^{-6}M and levelled off afterwards. The highest increases were observed with CPZ and CPZO, while those of PZ and PMZ were of lesser magnitude. However, the tested phenothiazines were without effect on kynurenine aminotransferase. The newly introduced psychotropic drug, sulpiride, which is a substituted benzamide, was devoid of activity on either enzyme both *in vivo* (50 mg/kg) and *in vitro* (3×10^{-9} to 3×10^{-4} M).

Psychoactive drugs of the phenothiazine series comprise one of the major tools in the management of neuropsychiatric disorders. Widespread use of this class of drugs warrants thorough investigation of their potential effects on metabolic pathways. The essential amino acid tryptophan, in addition to being an essential dietary component, is of importance as a precursor of the vitamin niacin and the biogenic amine serotonin, and is implicated in the regulation of enzyme levels and ribosome–polysome patterns. Metabolites of tryptophan are inhibitors of gluconeogenesis and oxidative phosphorylation. Some of these metabolites demonstrate carcinogenic activity in the mouse bladder [1]. Tryptophan has a variety of important metabolic pathways. From the standpoint of the chemical changes which occur, the so-called "kynurenine pathway" or "tryptophan–niacin pathway" is probably the most remarkable [2]. This pathway provides a convenient mechanism for the total degradation of the amino acid to non-aromatic products [3], with the eventual production of carbon dioxide and ammonia. All the suspected carcinogenic tryptophan metabolites are formed through this pathway. Most of the enzymatic reactions leading to the accumulation of these carcinogenic intermediates are vitamin B_6-dependent [4–7].

This study was planned to investigate the effect of some phenothiazines on the B_6-dependent kynurenine hydrolase and kynurenine aminotransferase, and also to compare the effect of these phenothiazines with the new psychotropic drug sulpiride, which is a substituted benzamide and bears no structural relationship to phenothiazines.

* Correspondence should be addressed to: Emad El-Bassiouni, Ph.D., Department of Pharmacology, Medical Research Institute, 165 El-Horreya Ave., P.O.B. El-Hadara, Alexandria, Egypt.

MATERIALS AND METHODS

Animals. Male albino mice were used in all experiments. The animals, kept under observation for one week before drug administration, weighed 25–30 g at the start of each experimental series.

Chemicals. The following drugs and chemicals were employed: chlorpromazine hydrochloride (CPZ) and chlorpromazine-5-oxide (CPZO) (Smith, Kline & French Laboratories, Philadelphia, PA); promazine hydrochloride (PZ) (Wyeth Laboratories Inc., Philadelphia, PA); promethazine (PMZ) (Misr Co. for Pharmaceutical Industries, Cairo, Egypt) and sulpiride (Delagrange International, Paris, France). L-Kynurenine sulphate and pyridoxal-5-phosphate were obtained from Sigma Chemical Co. (St. Louis, MO).

Treatment of animals. Drugs were dissolved in saline and different groups of mice were injected daily with either CPZ (5 mg/kg), PZ (10 mg/kg), PMZ (10 mg/kg) or sulpiride (50 mg/kg) for 5 consecutive days. Controls received an equal volume of the vehicle. The animals were killed by cervical dislocation 24 hr after the last injection.

Enzyme assays and quantitative determination of metabolites. Preparation of homogenates and incubation media were the same as previously described [8]. L-Kynurenine sulphate was used to bypass the tryptophan oxygenase step and to allow evaluation of kynurenine metabolism independently of any changes in oxygenase activity [9].

The incubation media and quantitative determination of kynurenine hydrolase and kynurenine aminotransferase activities were the same as described previously [10]. A semi-purified preparation of the enzymes was used [11]. The levels of enzyme activity were assessed by measuring the concentration of anthranilic and kynurenic acids produced from kynurenine hydrolase and kynurenine aminotransferase, respectively. Anthranilic acid was determined by the method of Mason and Berg [12]. Kynurenic acid was determined by the method of Satoh and Price [13]. The amounts of metabolites were expressed as μmoles/g liver. In the *in vitro* experiments, the drugs under study were added to the incubation tubes to give final concentrations of the drugs ranging from 3×10^{-9} up to 3×10^{-4} M. Student's *t*-test was performed on the data and probability values (P) of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

CPZ and other members of the phenothiazine family have been established as stimulators of drug metabolizing enzymes usually associated with hepatic microsomes [14–16]. Also, CPZ has been reported to induce certain enzymes involved in the activation and/or metabolism of some chemical carcinogens [17]. However, no attempt has been made to investigate the potential effect of phenothiazines on enzymes involved in the metabolism of kynurenine, which might eventually affect the production of some tryptophan metabolites suspected of being carcinogenic or co-carcinogenic [6, 7, 18–22].

In the present study, repeated daily administration of CPZ (5 mg/kg), PZ (10 mg/kg) and PMZ (10 mg/kg) to mice for 5 consecutive days resulted in increased activity of kynurenine hydrolase. The percentage increase over control value was 43, 36 and 25 for CPZ, PZ and PMZ respectively (Table 1). However, much higher doses of sulpiride (50 mg/kg) did not alter the activity of this enzyme (Table 1). The increase in the hydrolase activity might be due to conformational changes in the enzyme protein in the treated animals since CPZ and related compounds have high binding affinity for both proteins and the membrane lipids [23–25].

On the other hand, kynurenine aminotransferase responded differently to treatment with these phenothiazines. It was inhibited after 5 days of treatment. The inhibition was most apparent with CPZ (26%) and PMZ (22%). However, a mild but statistically significant decrease in activity was encountered with PZ (13%). Administration of sulpiride was without effect (Table 1).

The difference in the response of the two enzymes to the applied phenothiazines parallelled other data shown earlier [26–29], using pyridoxine antimetabolites.

In vitro experiments were planned in which the applied phenothiazines and sulpiride were directly added to the incubation medium in order to assess the direct effect of these compounds on kynurenine hydrolase and kynurenine aminotransferase. In the presence of CPZ, CPZO, PZ and PMZ, it was observed that the higher the drug concentration the higher was the activity of kynurenine hydrolase, up to 3×10^{-6} M drug concentration. Increasing drug concentration beyond 3×10^{-6} M was ineffective in further increasing the activity of the enzyme. The highest activation was observed with CPZ and CPZO (66%) followed by PZ and PMZ (about 30%) which is the same sequence observed in the *in vivo* experiments. It is noteworthy that CPZO, which is the major metabolite of CPZ and which is pharmacologically inactive, produced the same magnitude of activation as CPZ. It seems, therefore, that the presence of the phenothiazine nucleus is an essential property to induce the activation of the kynurenine hydrolase by these phenothiazine derivatives. This activation effect could be attributed to the presence of an active group (*s*) in the structure of these phenothiazines which may facilitate the binding of these compounds to different regions on the enzyme protein. Such binding may consequently lead to an increased affinity of the apoenzyme to its cofactor and/or substrate.

Despite the mild inhibition of kynurenine aminotransferase observed in the *in vivo* treatment, the phenothiazines under test were without effect when added to the incubation media in the concentration range tested.

Sulpiride did not affect either enzyme either *in vivo* or *in vitro*.

Table 1. *In vivo* effect of phenothiazines and sulpiride on kynurenine metabolism in mouse liver homogenates

Treatment	No. of mice	Kynurenine hydrolase (μmoles anthranilic acid/g liver)* Control	Kynurenine hydrolase (μmoles anthranilic acid/g liver)* Experiment	Kynurenine aminotransferase (μmoles kynurenic acid/g liver)* Control	Kynurenine aminotransferase (μmoles kynurenic acid/g liver)* Experiment
CPZ (5 mg/kg)	8	1.21 ± 0.20	1.73 ± 0.21† (43% increase)	4.11 ± 0.29	3.06 ± 0.17† (26% decrease)
PZ (10 mg/kg)	6	1.18 ± 0.05	1.60 ± 0.19† (36% increase)	4.20 ± 0.34	3.66 ± 0.22† (13% decrease)
PMZ (10 mg/kg)	6	1.18 ± 0.05	1.48 ± 0.07† (25% increase)	4.20 ± 0.34	3.26 ± 0.31† (22% decrease)
Sulpiride (50 mg/kg)	8	1.21 ± 0.20	1.13 ± 0.13 (No effect) N.S.	4.11 ± 0.29	4.34 ± 0.66 (No effect) N.S.

* Results are expressed as mean ± S.D.
† Values are significantly different from corresponding control values ($P < 0.05$).
N.S. Statistically not significant.

Table 2. *In vitro* effect of phenothiazines and sulpiride on kynurenine metabolism in mouse liver

Drug conen	Kynurenine hydrolase (μmoles anthranilic acid/g liver)*	% Activation	Kynurenine aminotransferase (μmoles kynurenic acid/g liver)*
None	1.09 ± 0.11	—	4.33 ± 0.26
CPZ			
3×10^{-9} M	1.17 ± 0.12	7	4.40 ± 0.31
3×10^{-7} M	1.32 ± 0.03†	21	4.38 ± 0.21
3×10^{-6} M	1.80 ± 0.03†	65	4.47 ± 0.30
3×10^{-5} M	1.83 ± 0.08†	68	4.43 ± 0.11
3×10^{-4} M	1.82 ± 0.05†	67	4.45 ± 0.15
CPZO			
3×10^{-9} M	1.20 ± 0.08	10	4.38 ± 0.21
3×10^{-7} M	1.36 ± 0.04†	25	4.36 ± 0.23
3×10^{-6} M	1.77 ± 0.06†	62	4.50 ± 0.10
3×10^{-5} M	1.83 ± 0.08†	68	4.37 ± 0.15
3×10^{-4} M	1.83 ± 0.06†	68	4.33 ± 0.32
PZ			
3×10^{-9} M	1.14 ± 0.08	5	4.33 ± 0.24
3×10^{-7} M	1.20 ± 0.07	10	4.43 ± 0.18
3×10^{-6} M	1.47 ± 0.13†	35	4.45 ± 0.14
3×10^{-5} M	1.35 ± 0.04†	28	4.50 ± 0.18
3×10^{-4} M	1.49 ± 0.09†	37	4.52 ± 0.22
PMZ			
3×10^{-9} M	1.11 ± 0.08	—	4.49 ± 0.27
3×10^{-7} M	1.17 ± 0.07	7	4.47 ± 0.25
3×10^{-6} M	1.40 ± 0.08†	28	4.52 ± 0.17
3×10^{-5} M	1.49 ± 0.05†	37	4.53 ± 0.15
3×10^{-4} M	1.43 ± 0.05†	31	4.57 ± 0.20
Sulpiride			
3×10^{-9} M	1.11 ± 0.11	No effect	4.52 ± 0.31
3×10^{-7} M	1.05 ± 0.09	No effect	4.37 ± 0.16
3×10^{-6} M	1.07 ± 0.04	No effect	4.50 ± 0.24
3×10^{-5} M	1.06 ± 0.06	No effect	4.25 ± 0.25
3×10^{-4} M	1.08 ± 0.05	No effect	4.11 ± 0.28

* Each value represents the mean value of 5 experiments ± S.D.
† Statistically different from control ($P < 0.05$).

It has been emphasized that under certain conditions, quite low concentrations of phenothiazines can have marked effects on tissues and membranes, but these effects probably have little or no relationship to their pharmacological action [30]. In our case, it seems that the applied compounds with the phenothiazine nucleus may induce disordered kynurenine metabolism, at least in part, by direct action on its metabolizing enzymes. This, in turn, might affect NAD biosynthesis and/or the accumulation of some tryptophan metabolites suspected to be carcinogens or co-carcinogens [6, 7, 18–22].

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