Biochemical Pharmacology, Vol. 39, No. 5, pp. 827–832, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

THE INHIBITION OF GLUTAMATE DEHYDROGENASE BY SOME ANTIPSYCHOTIC DRUGS

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(*Received* 24 *April* 1989; *accepted* 15 *September* 1989)

Abstract—The phenothiazines chlorpromazine and perphenazine and the butyrophenone haloperidol were shown to be reversible inhibitors of glutamate dehydrogenase (GDH). Inhibition by chlorpromazine was found to be partial, whereas haloperidol and perphenazine would, apparently, give full inhibition at saturating concentrations. Double-reciprocal plots of the difference between activities in the absence and presence of the inhibitor against the inhibitor concentration were linear with chlorpromazine and perphenazine but parabolic when haloperidol was used as the inhibitor. Comparisons between the responses of commercially available preparations of ox liver GDH, which have been shown to have suffered limited proteolysis during purification, and the ox brain enzyme prepared by a procedure which does not result in such proteolysis, revealed the latter preparation to be more sensitive to inhibition because of a higher apparent affinity for these drugs. The apparent dissociation constants for enzyme–drug interactions were, however considerably higher than the concentrations that have been reported to occur *in vivo* following chronic administration of chlorpromazine and haloperidol. This casts doubt on earlier claims that inhibition of GDH may be involved in the antipsychotic actions of these drugs, although it might be a factor in the side effects associated with the use of such compounds.

Phenothiazines and butyrophenones have been shown to be inhibitors of glutamate dehydrogenase (L-glutamate:NAD(P)$^+$ oxidoreductase (deaminating); EC1.4.1.3, GDH) activity [1]. Furthermore the relative potencies of several such compounds as inhibitors of the enzyme were found to correlate with their relative pharmacological potencies as antipsychotic drugs [1] and a similar correlation was found when their binding to purified ox liver GDH was determined [2]. The interaction between glutamate dehydrogenase and antipsychotic drugs appears to be specific since a variety of other drugs were found to be without effect on the activity of the enzyme [3]. These results led to the suggestion that the interaction with GDH was a potentially important aspect of the action of these drugs [1, 2]. Whatever the relationship between this phenomenon and the antipsychotic actions of the phenothiazines and butyrophenones, it appears that it cannot be ignored in any attempts to understand their biochemical and pharmacological actions.

It has been found, more recently, that commonly-used preparations of glutamate dehydrogenase had suffered limited proteolytic digestion during the purification procedures used [4] and that this resulted in some changes in its behaviour with substrates, inhibitors and activators [5–8]. Because of this we have reinvestigated the interactions of the enzyme with these drugs using purified, apparently homogeneous, preparations of the enzyme from ox brain, which have been shown not to have suffered limited proteolysis. Perphenazine and chlorpromazine were used as examples of the phenothiazines whereas haloperidol was used as a representative butyrophenone.

MATERIALS AND METHODS

Enzyme preparation. Ox brain GDH was purified to apparent homogeneity and shown not to have suffered limited proteolytic cleavage during the preparation by the procedures previously described [4]. Purified preparations of ox liver GDH, which have been shown to have suffered limited proteolytic cleavage [4], were obtained from Boehringer Corp. (Mannheim, F.R.G.) or Sigma Chemical Co. (Poole, U.K.).

Enzyme assay. Enzyme activities were determined spectrophotometrically by monitoring the oxidation of NADH at 340 nm. Unless otherwise stated assays were carried out at 30° in a mixture containing, in a total volume of 2.5 mL, 50 mM phosphate buffer, pH 7.4, 100 mM NH_4Cl, 80 μM NADH, the enzyme sample at a final concentration within the range 0.05–0.4 μg/mL and 5 mM 2-oxoglutarate, which was added to start the reaction.

Effects of inhibitors. Chlorpromazine and perphenazine (from Sigma) were dissolved in small volumes of 0.05 M HCl and the solutions were adjusted to pH 7.0. The contents of ampoules of haloperidol in aqueous solution (Searle) were adjusted to pH 5.0 with 5 M NaOH to give a final stock solution concentration of 13 mM. All inhibitor solutions were made up freshly each day, kept on ice and protected from exposure to light. Where appropriate data were plotted graphically as $1/(V_i - v)$ vs $1/[M]$ as described by Shemisa and Fahien [1] where V_i is the velocity in the absence of inhibitor, v is the velocity in its presence and $[M]$ is the concentration of the inhibitor. These plots were

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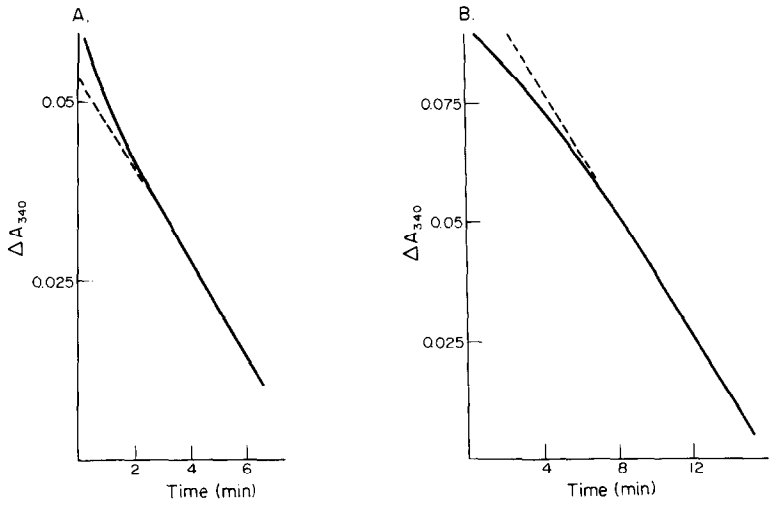

Fig. 1. Time-courses of the reductive amination of 2-oxoglutarate catalysed by GDH in the presence of perphenazine. The reaction was followed spectrophotometrically as described in the text except that it was started by the addition of the ox brain enzyme sample. (A) GDH was preincubated in buffer for 5 min at 30° before addition to the reaction mixture which contained 45 μM perphenazine. (B) The enzyme was preincubated under the same conditions with 225 μM perphenazine before dilution 5-fold into the assay mixture which contained no additional perphenazine. In both cases the final concentration of GDH in the assay mixture was 0.08 μg/mL. The final steady-state rates, corresponding to the broken lines, were 13.2 μmol/min/mg protein (A) and 13.1 μmol/min/mg protein (B).

evaluated using the following relationship [1], which is appropriate for both full and partial inhibitors:

$$1/(V_1 - v) = 1/(V_1 - V_2) + K_3/(V_1 - V_2)[M]$$

where V_2 is the velocity in the presence of saturating concentrations of inhibitor and K_3 is the concentration of effector necessary to give $v = (V_1 + V_2)/2$. Data were evaluated by the direct fitting of the dependence of $(V_1 - v)$ on $[M]$ by non-linear regression to give values of K_3 and $V_1 - V_2$. Fieller's theorem (see Ref. 9) was used to estimate the standard deviation for V_1/V_2.

The reversibility of inhibition was monitored by dilution of preincubated drug–enzyme samples. GDH was preincubated for 5 min at 30° in the presence and absence of the drug at a concentration of $5x$ M. At the end of the preincubation period 0.5-mL aliquots were added to 2.0 mL reaction mixture. For samples that had been incubated in the absence of inhibitor the mixture also contained x M inhibitor whereas there was no inhibitor in the assay mixture to which enzyme samples incubated in the presence of inhibitor were added. Thus in both cases the final drug concentration was x M.

RESULTS

Time-courses of inhibition

The addition of the enzyme to the reaction mixture containing the antipsychotic drug resulted in an initial rapid decrease in the reaction velocity after which the progress curve was linear (Fig. 1).

When the final, linear, rate of reaction was determined at a series of inhibitor concentrations chlorpromazine, perphenazine and haloperidol were found to inhibit the activity of GDH. Preincubation of the enzyme with these inhibitors for periods of up to 10 min showed no time-dependent changes in the effects on the steady-state rate.

Reversibility

The reversibility of the inhibition was assessed by dilution. As shown in Fig. 2 the inhibition by perphenazine was freely reversible by this procedure. However, as shown in Fig. 1B, an initial lag phase was evident, before the steady-state progress curve was established, when the enzyme–perphenazine mixture was added to the assay system. This suggests that reversibility of the inhibition is a relatively slow process although it proceeded to completion.

Kinetics of the inhibition of native and proteolysed GDH

As shown in Fig. 2 the preparation from ox liver, which had suffered limited proteolysis during the purification procedure, was less sensitive to inhibition by perphenazine than the ox brain preparation. Such differences were investigated in more detail by examining the effects of varying concentrations of chlorpromazine, perphenazine and haloperidol on the steady-state rates of the reductive amination of 2-oxoglutarate. In these studies the activities of the proteolysed ox liver enzyme and those of the unproteolysed brain GDH were assayed alternately (see Ref. 7) to allow the most accurate comparison of the responses of the two preparations.

Chlorpromazine (Fig. 3), perphenazine (Fig. 4) and haloperidol (Fig. 5) were each found to be more potent inhibitors of the unproteolysed ox brain preparation than of the proteolysed liver GDH. In order to determine the kinetic factors responsible for this difference the data were analysed by non-linear

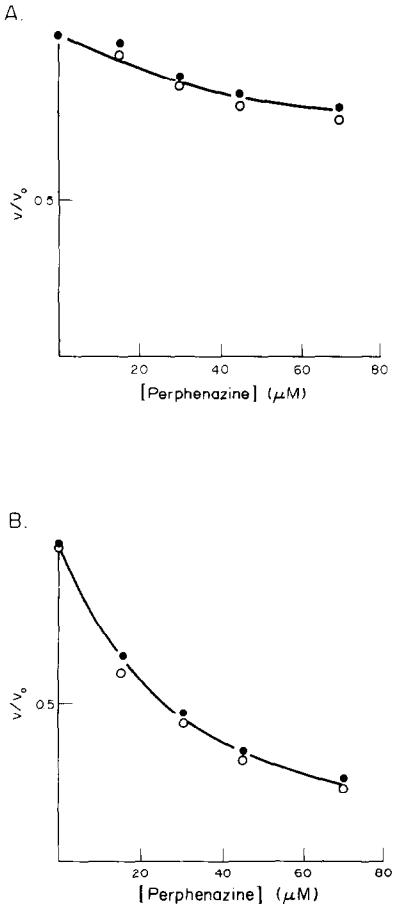

Fig. 2. Reversibility of the inhibition of GDH by perphenazine. Ox liver GDH (Sigma), which had suffered limited proteolysis during the purification procedure (A) and ox brain GDH, which had not (B) were preincubated at 30° either without perphenazine (○) or with 5 x M perphenazine (●). After 5 min 0.5-mL aliquots were added to the assay mixture (2.0 mL) containing either x M (○) or no additional (●) perphenazine. Thus the concentration of perphenazine in the assay was x M in both cases. v and v_0 are, respectively, the velocity in the presence and absence of perphenazine. The results are presented as a plot of the ratio v/v_0 vs the final concentration of perphenazine in the assay. Each experimental point is the mean of duplicate or triplicate velocity determinations.

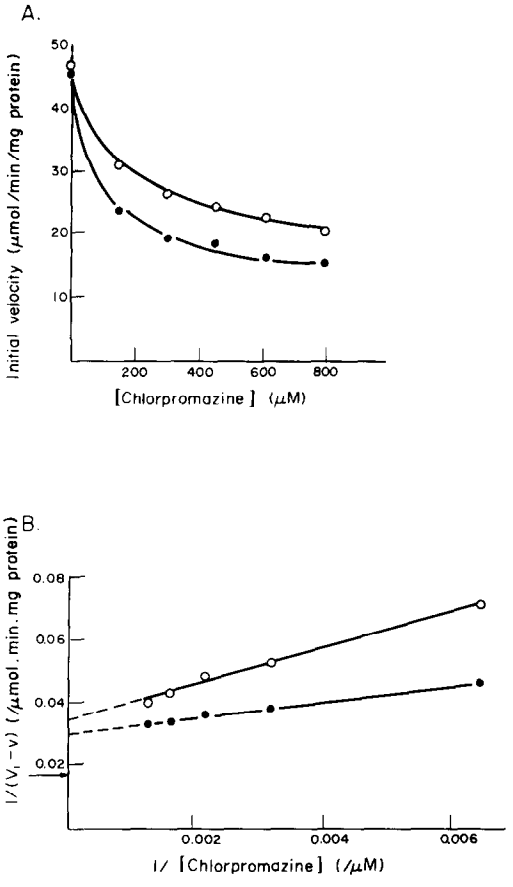

Fig. 3. The effects of chlorpromazine on GDH activity. Ox liver GDH, obtained from Boehringer Corp., (○) and ox brain GDH, prepared by a procedure [4] that does not result in proteolysis occurring during the purification, (●) were assayed alternately as described in the text. (A) The dependence of the steady-state velocity on the concentration of chlorpromazine. Each point represents the mean of duplicate determinations. (B) Plot of $1/(V_1 - v)$ vs the reciprocal of the chlorpromazine concentration, as described in the text. K_3 and V_1/V_2 values for the ox liver preparation were 180 μM (±20, SE) and 2.9 (±0.4, SE), respectively. The corresponding values for the laboratory-purified ox brain enzyme were 84 ± 7 μM and 3.6 ± 0.4. The arrow indicates the value of $1/V_1$.

regression to allow K_3 and V_1/V_2 to be determined. Double-reciprocal plots of the data are shown in Figs 3B, 4B and 5B.

Linear double-reciprocal replots were obtained with chlorpromazine and perphenazine whereas an upward curvature was observed when haloperidol was used (Fig. 5B). In that case apparent values for K_3 and V_1/V_2 were estimated from experimental data obtained with haloperidol concentrations higher than 15 μM.

In the case of inhibition by chlorpromazine the value of $V_1 - V_2$ was found to be significantly different from V_1 indicating the inhibition to be partial. The inhibition by high concentrations of perphenazine and haloperidol was, however, complete. The values for the constants determined in this way are shown in Table 1 and individual standard error estimates from the curve-fitting are given in the legends to Figs 3–5. Values of V_1/V_2 for inhibition by perphenazine and haloperidol showed no detectable differences between the ox brain and liver preparations. Thus the difference between the sensitivities of the two enzyme preparations to inhibition by these antipsychotic drugs depends on their affinities for inhibitor binding, as measured by the constant K_3. In the case of chlorpromazine, however, the unproteolysed preparation from ox brain was somewhat more sensitive to inhibition than the ox liver preparation which had suffered proteolysis.

DISCUSSION

Detailed comparisons of ox liver and brain glutamate dehydrogenase preparations which have not

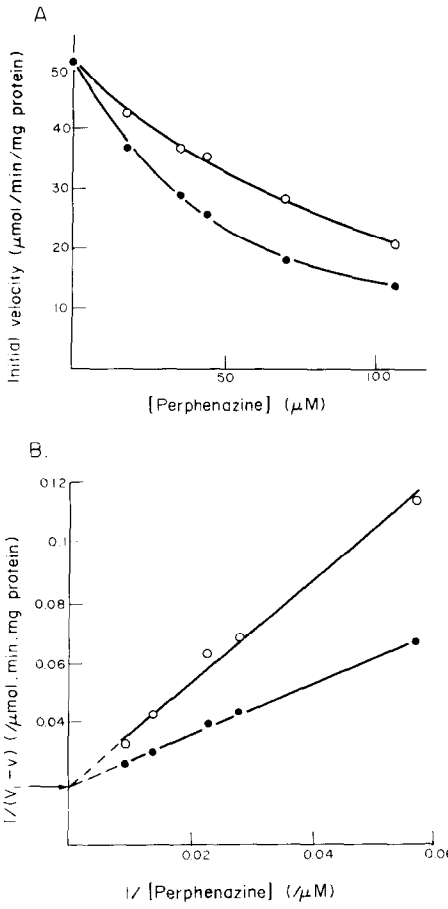

Fig. 4. The effects of perphenazine on GDH activity. Details were as described in the legend to Fig. 3. The K_3 and V_1/V_2 values for the ox liver enzyme preparation (○) were determined to be 110 μM (±20, SE) and >100, respectively, and those for the laboratory-purified ox brain preparation (●) were 50 ± 4 μM and >100, respectively.

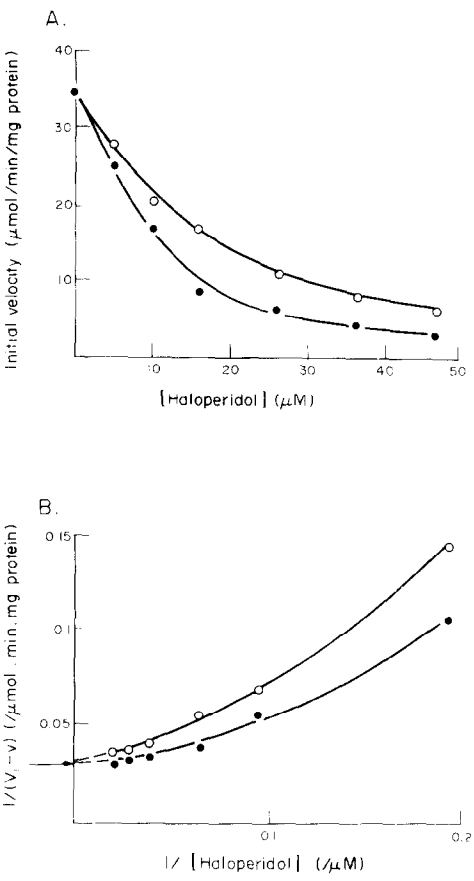

Fig. 5. The effects of haloperidol on GDH activity. Details were as described in the legend to Fig. 3. The values of K_3 and V_1/V_2 for the ox liver enzyme preparation were estimated from the experimental data obtained for haloperidol concentrations greater than 15 μM to be 14 μM (±1, SE) and >100, respectively. The values for the laboratory-purified ox brain enzyme were estimated, in the same way, to be 5.3 ± 0.5 μM and >100, respectively.

suffered proteolysis during the purification procedure have not revealed any structural or behavioural differences between them [4–7]. Thus the differences in the behaviour of ox liver and brain GDH reported here appear to result from the limited proteolysis of the former preparation decreasing its affinity for these antipsychotic drugs. The limited proteolysis that can occur during purification results in the loss of the N-terminal four amino acids and has also been shown to affect the affinity of the enzyme for allosteric effectors [5–8].

The ox brain and liver GDH preparation used by Shemisa and Fahien [1] in their studies of the effects of antipsychotic drugs was prepared by a procedure involving successive precipitation and crystallization steps [10] and it appears that such a method would result in limited proteolysis [4, 5]. Thus those enzyme samples should resemble the proteolysed ox liver preparation used in the present work. The order of inhibitory potency of the drugs used in the present study (Table 1) was similar to that reported in the earlier studies [1]. However there are some other notable differences. Shemisa and Fahien [1] reported both perphenazine and haloperidol to be partial inhibitors of GDH whereas the data obtained here were consistent with complete inhibition. Also the absolute values for K_3 obtained in the earlier study differed from those obtained in this work (Table 1). The assay conditions used in these two studies were, however, not the same and the kinetic behaviour has been shown to vary with different buffer conditions [11].

Although the inhibition of GDH by the antipsychotic drugs used in this study appeared to be freely reversible there was an initial lag period before the steady-state rate was achieved when samples of enzyme which had been preincubated with perphenazine were added to the assay mixture (Fig. 1). Shemisa and Fahien [1] also reported the existence of a lag phase under similar experimental conditions. Conversely when the reaction was started by the addition of enzyme to an assay mixture containing perphenazine there was an initial burst before the steady-state was reached. Hysteretic behaviour in the reaction of GDH with other ligands have also

Table 1. The inhibition of glutamate dehydrogenase preparations by chlorpromazine, perphenazine and haloperidol

Enzyme preparation	Chlorpromazine		Perphenazine		Haloperidol	
	K_3 (μM)	V_1/V_2	K_3 (μM)	V_1/V_2	K_3 (μM)	V_1/V_2
Ox brain (not proteolysed)	70 ± 15	3.6 ± 0.1	48 ± 3	>100	5.2 ± 0.1	>100
Ox liver (proteolysed)	150 ± 30	2.8 ± 0.1	120 ± 40	>100	13.6 ± 0.4	>100
Ox liver (taken from Ref. 1)	32	6	20	50	40	100

Values for the kinetic parameters were determined by the procedures described in the text. All values determined in this work are means, ± range, from duplicate determinations except for the values for perphenazine inhibition of the ox liver preparation where triplicate determinations were made and the value for K_3 is the mean ± SE. The standard errors obtained from curve-fitting to obtain the individual values of the parameters are given in the legends to Figs 3–5. There were no error estimates provided with the data of Shemisa and Fahien [1].

been reported [12] and may represent slow conformational transitions associated with such interactions.

The steady-state concentrations of haloperidol in human blood have been determined in patients receiving doses of 5–20 mg per day [13] to be 5–10 μM both in plasma and erythrocyte. Similarly for patients receiving 900 mg of chlorpromazine per day the plasma and erythrocyte levels were 0.64 and 0.36 mM, respectively [14]. In the rat the plasma level of chlorpromazine in rats receiving 2 × 2 mg/kg doses per day the concentrations in plasma were 15 μM whereas those in liver and brain were 40 and 100 μmol/kg tissue, respectively [15]. These plasma and erythrocyte values are much lower than the apparent dissociation constants for enzyme inhibition (Table 1). However, chlorpromazine accumulates to higher concentrations in brain and liver than in blood. Furthermore it is possible that such compounds might become concentrated within the mitochondrion.

Although a number of drugs which do not have antipsychotic actions have been shown not to inhibit GDH [2], a range of other compounds, including steroids, thyroxine, anionic dyes [see 16, 17] and gossypol [18] have been shown to be inhibitors of the enzyme. Thus it appears that inhibition of glutamate dehydrogenase is not a specific property necessary for the action of antipsychotic drugs. The possibility that inhibition of this enzyme may contribute to some of the effects seen on short or long term administration of these compounds cannot, however, be excluded.

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