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Modifications of Glutamate Dehydrogenase by Various Drugs Which Affect Behavior

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SUMMARY

The studies presently reported are consistent with the concept that chlorpromazine, certain isosteres of phenothiazine, and haloperidol (all drugs known to affect behavior) are inhibitors of glutamate dehydrogenase. These drugs apparently are bound to a site on the enzyme distinctly different from the active or purine nucleotide-binding sites. A lysine group on the enzyme molecule which is quite reactive with pyridoxal phosphate and is essential for full enzyme activity and allostery does not seem to be part of the drug-binding site. However, after this lysine group has reacted with pyridoxal phosphate, chlorpromazine will not inhibit the enzyme.

Since binding of these drugs alters the absorption spectrum, fluorescence, and sedimentation coefficient of the enzyme, it is believed that inhibition by these drugs is secondary to an induced change in the conformation of the enzyme. In general the drugs are more inhibitory if the enzyme is already in a rather inactive conformation, i.e., if high concentrations of DPNH or GTP are present, and much less inhibitory if the enzyme is in an activated conformation, i.e., in the presence of high concentrations of ADP or DPN. Therefore the conformational changes produced by the drugs seem to be enhanced in the inactivated enzyme and retarded in the activated enzyme.

In the presence of TPNH the drugs have little effect unless GTP is present. The drugs are extremely inhibitory in the presence of DPNH only if the concentration of DPNH is sufficiently high to produce substrate inhibition. ATP does not markedly alter the inhibitory action of the drugs with either coenzyme, and none of the drugs is a potent inhibitor in the presence of ADP or DPN. Therefore these agents would be most effective as inhibitors of ammonia incorporation if the DPNH:DPN and ATP:ADP ratios are high or if the concentrations of both TPNH and GTP are high.

A detailed structure-activity study of the effects of many isosteres of promazine on glutamate dehydrogenase was performed, and the essential parts of the drug molecule necessary for binding to the drug site were characterized. The effects of these isosteres on glutamate dehydrogenase correlate closely with their antipsychotic activity *in vivo*.

INTRODUCTION

It has previously been shown that some drugs which are known to affect behavior, such as chlorpromazine and isosteres of phenothiazine, are inhibitors of glutamate dehydrogenase (EC 1.4.1.3) (1). Furthermore, these results were consistent with the concept that chlorpromazine is bound to an allosteric site on the enzyme different from that binding the purine nucleotide or the active sites.

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The level of glutamate dehydrogenase in brain is high (2, 3). The inhibition constant of chlorpromazine in the glutamate dehydrogenase reaction is about 10 times lower than estimates of the pharmacological levels of this drug in brain (1, 4). Chlorpromazine can enter mitochondria and is known to increase brain levels of glutamine (5, 6). Glutamate is known to play an important role in the central nervous system (7–9). Consequently, it has been suggested that the effects of chlorpromazine on glutamate dehydrogenase could be related to the pharmacological action of this drug. In this paper additional studies of the effects of chlorpromazine and several other drugs on glutamate dehydrogenase are reported.

MATERIALS AND METHODS

Enzymes and reagents. Bovine glutamate dehydrogenase was prepared by methods described previously (10). The kinetic properties described in this paper are the same for both the bovine brain and liver enzymes. Coenzymes were obtained from P-L Biochemicals, and other substrates from Sigma Chemical Company. The structures of the drugs used are given in Table 4. Phenothiazine base and 2-chlorophenothiazine base were purchased from Aldrich Chemical Company. Drugs were generously supplied by Smith Kline & French [SKF 2680, chlorpromazine (Thorazine), chlorpromazine sulfoxide, triflutrimeprazine (SKF 5354), and prochlorperazine (Compazine)], Geigy Pharmaceuticals [imipramine (Tofranil) and desipramine (Pertofrane)], Riker Laboratories [orphenadrine (Disipal)], the Squibb Institute (trifluprομazine), G. D. Searle & Company [aldactone and fenethazine (SC 1627)], the Merck Institute (hydrochlorothiazide), Schering (perphenazine), Wyeth Laboratories (promazine and promethazine), Lederle Laboratories [methoxypromazine (Tentone)], Rhone Poulenc [diethazine (RP 2989) and chlorproethazine (RP 4909)], McNeil Laboratories [haloperidol (Haldol)], Lake Side Laboratories and Dr. Albert Manion of the National Institutes of Health [demonomethyl chlorpromazine, dedimethylchlorpromazine, EX 10-029 (Lake Side Laboratories experimental antiparkinsonian compound)], Chas. Pfizer & Company (doxepin), and Merck (amitriptyline). These drugs were sufficiently soluble in water or in 0.01 N HCl to be used in our experiments. The concentrations of drugs used in the assays were low, so that additions to the enzyme assay mixture did not alter the pH. Solutions of the drugs were made fresh, protected from exposure to light, and stored at 4°.

Initial velocity measurements. Enzyme assays were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°. The enzyme was dialyzed against this buffer before use. Reaction rates were followed spectrophotometrically by measuring the rate of oxidation of DPNH or TPNH or the reduction of DPN at 340 or 360 mμ (when high concentrations of DPNH were used), using a Gilford model 2000 recorder and a Beckman DU Monochromator. Corrections were made for loss of enzyme activity by performing standard assays periodically during the course of the kinetic experiments (10).

Since many of the drugs used in these experiments absorb light at 340 mμ, control experiments were performed at 340 mμ in the absence of enzyme, and in the presence of enzyme but absence of coenzyme. None of the drugs produced changes in absorbance in control experiments, and were found to be stable during the time required to measure initial velocities. Kinetic experiments were performed at least three times, and experimental points represent average values. These experiments were found to be essentially completely reproducible. The standard assay mixture referred to throughout the text consisted of 2 mM α-ketoglutarate, 50 mM NH_4Cl, and 100 μM DPNH in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

Protein concentration. The concentration of glutamate dehydrogenase was determined by measuring the absorbance at 280 mμ, using 0.97 cm^2 mg^{-1} (11) as the extinction coefficient.

Ultracentrifugation. Sedimentation experiments were performed in a Spinco model E analytical centrifuge at 20°.

Absorption spectra. Absorption spectra were measured either with a Beckman DU Monochromator or with a Cary model 14 automatic recording spectrophotometer.

Fluorescence measurements. These experi-

ments were performed at 25° in 0.025 M sodium arsenate, pH 7.8, with 0.1 mM EDTA in an Aminco-Bowman spectrophotofluorometer equipped with a thermoelectric cooler, a Glans prism polarizer, and an X-Y recorder. In fluorescence experiments in which the enzyme was titrated with drugs, corrections were made for dilution. Also, since the drugs used in these experiments absorb light at 280 mμ (the exciting wavelength), control experiments were performed by adding the drug to a solution of tryptophan which was adjusted so that the intensities of emitted fluorescence from the tryptophan and enzyme solutions were equal. Therefore the results represent the difference between the effects of the drug on glutamate dehydrogenase fluorescence and on tryptophan fluorescence. It was assumed that the decrease in tryptophan fluorescence produced by the drug was due to internal quenching of the exciting light by the drug, and not to an interaction between tryptophan and the drug.

KINETIC THEORY

It has previously been demonstrated that chlorpromazine in the absence of purine nucleotides inhibits glutamate dehydrogenase mainly by increasing the amount of substrate inhibition by DPNH. Substrate inhibition could result from binding of DPNH to two distinctly different sites (active and modifier) on each peptide chain of the enzyme (12), with binding to the modifier site resulting in inhibition of the active site. Alternatively, each peptide chain on the enzyme might possess one potentially equal, active DPNH-binding site, and negative interaction would take place among these sites. These interactions would result in a progressive increase in the dissociation or Michaelis constant for DPNH and inhibition of the enzyme reaction. Kinetic data alone cannot distinguish between these two, or several other, possibilities. When the concentration of enzyme is high, there is no evidence of substrate inhibition by DPNH, and studies of the binding of DPNH to glutamate dehydrogenase are consistent with there being six equal, noninteracting DPNH-binding sites per enzymatically active monomer (mol wt 2.8×10^5) (13–17). This is the same number of sites found for other coenzymes and purine nucleotides which show binding of the Michaelis-Menten type when the enzyme concentration is low (18). When the concentration of enzyme is high (as in binding experiments), the enzyme associates to polymeric forms (18). The loss of substrate inhibition which occurs at high enzyme concentration could result either from a masking of the DPNH modifier site by the polymerized enzyme or from a loss of interaction between chains in the polymerized enzyme.

When the concentration of enzyme is low, the effect of DPNH on the initial velocity of DPNH oxidation (v) with respect to DPNH concentration (A) can be expressed by Eq. 1 (19).

$$v = \frac{k_1[E_0]}{1 + \frac{K_1}{(A)} + \frac{(A)}{K_2}} \qquad (1)$$

If there are two types of binding sites (active and modifier), then K_1 is the Michaelis constant for DPNH at the active site, K_2 is the dissociation constant at the modifier site, and $k_1[E_0]$ is the maximal velocity. The general equation for an enzyme with six interacting sites (20) can be simplified to a form empirically identical with Eq. 1 if there are certain relationships between intrinsic dissociation constants K_j and average catalytic rate constants per site k_j for a particular complex.[2] For example, if $K'_2 = 5K'_6$, $K'_3 = 0.33K'_1$, $K'_4 = 0.6K'_6$, $K'_5 = K'_1$, $k_2 = k_4 = k_6 = 0$, $k_3 = 0.33k_1$, and $k_5 = 0.2k_1$, then the general equation for six interacting sites becomes

$$v = \frac{k_1[E_0]}{1 + K'_1/6(A) + (A)/K'_6(6)} \qquad (2)$$

Since Eq. 2 cannot be distinguished by kinetic experiments from Eq. 1, kinetic results in this paper were evaluated with Eq. 1.

Kinetic experiments performed with vari-

[2] The subscripts refer to the intrinsic dissociation or rate constant for a particular complex. For example, the constant K'_6 refers to the intrinsic dissociation constant of the complex E-(DPNH)$_6$ (20).

ous concentrations of drugs were evaluated with the use of Eq. 3.

$$(V_1 - v) = \frac{V_1 - V_2}{1 + K_3/(M)} \quad (3)$$

where V_1 is the velocity in the absence of a drug, v is the velocity in the presence of a drug, V_2 is the velocity in the presence of saturating concentrations of the drug; K_3 is the apparent dissociation constant of the drug, and (M) is the concentration of the drug. The constants V_2 and K_3 were estimated from double-reciprocal plots of the change in velocity produced by the drug $(V_1 - v)$ with respect to drug concentration as described previously (1). In some experiments these double-reciprocal plots were not linear. The results were evaluated with the use of Eq. 4.

$$(V_1 - v) = \frac{V_1 - V_3}{1 + K_3/(M) + K_3K_4/(M)^2} \quad (4)$$

This is a general equation for a modifier which is bound poorly at low concentrations to one or a few equally available sites (with dissociation constant K_3); binding to this site(s) enhances binding to the additional site(s) (with a dissociation constant K_4). Binding to the loose site(s) (expressed by K_3) produces essentially no change in velocity, but binding to the tight sites (expressed by K_4) produces a change in velocity. V_1 has been defined previously, and V_3 is the velocity when the enzyme is saturated with drug. Equations empirically similar to Eq. 4 can be obtained by straightforward simplifications of the general equation for the effect of a modifier on an enzyme with interacting sites (20). However, results compatible with Eq. 4 could also be obtained if there are two distinctly different drug-binding sites on each chain. Again, one cannot distinguish between these two possibilities on the basis of kinetic data alone.

RESULTS

Kinetics

Chlorpromazine. The effects of chlorpromazine on the rate of oxidation of DPNH or TPNH in the presence or absence of purine nucleotides are summarized in Tables 1[3] and 2. These kinetic constants were calculated from experimental results similar to those shown in Figs. 1 and 2. In the absence of purine nucleotides, chlorpromazine inhibits the oxidation of DPNH more than that of TPNH, mainly because chlorpromazine increases the amount of substrate inhibition by DPNH (chlorpromazine decreases the value of K_2 in Table 1 and Eq. 1). The dissociation or inhibition constant of chlorpromazine is lower when the concentration of DPNH is sufficiently high to produce substrate inhibition (Table 2). In the presence of ATP, chlorpromazine has little effect on TPNH oxidation (the dissociation constant of the drug exceeds 200 μM), but in the presence of DPNH plus ATP chlorpromazine is inhibitory, since saturating concentrations of the drug inhibit 100-fold (Table 2). In the presence of GTP, chlorpromazine inhibits more when TPNH is the coenzyme, since k_1 is decreased 2-fold in the presence of TPNH (Table 1), while in the presence of DPNH plus GTP the dissociation constant of the drug is greater than 200 μM (Table 2). In the presence of ADP, chlorpromazine has little effect on the reaction with either reduced coenzyme, since in both cases the dissociation constant of the drug is high (Table 2) and ADP almost eliminates substrate inhibition by DPNH (Table 1).

Perphenazine. In the absence of purine nucleotides, perphenazine, like chlorpromazine, increases substrate inhibition by

[3] These results, obtained in the presence of arsenate buffer and absence of drugs, are slightly different from those previously reported when Tris-acetate was used as a buffer (12). The main difference between arsenate or phosphate and Tris-acetate is that in the presence of the latter there is more substrate inhibition by DPNH (21, 22). Results obtained with chlorpromazine in Tris-acetate are qualitatively similar to those with arsenate, except that the value of K_3 is slightly lower in the presence of Tris-acetate. This is consistent with the concept that chlorpromazine has its main effect when substrate inhibition by DPNH is greater, i.e., in this case, in the presence of Tris-acetate. The values given for K_2 are only estimates above 0.3 mM, because of the technical difficulties caused by high concentrations of DPNH.

DPNH (Fig. 1 and Table 1). Inhibition by perphenazine is likewise markedly decreased by ADP but not by GTP or ATP (Figs. 2 and 3 and Tables 1 and 2). Unlike chlorpromazine, however, perphenazine displays increased inhibitory effects if incubated with the enzyme for about 4 min before the assay. More inhibition is produced if a high concentration of glutamate dehydrogenase is incubated with perphenazine and then diluted and assayed than if a smaller amount of enzyme (that required for optimal assay) is incubated with the same amount of drug (Fig. 4, curve *C* vs. *D*). However, after about 2 min the velocity of the diluted sample (Fig. 4, curve *D*) begins to increase and ap-

TABLE 1

Effects of chlorpromazine and perphenazine on kinetic constants for DPNH and TPNH in the glutamate dehydrogenase reaction

The kinetic constants are those used in Eq. 1. Experimental conditions are described in the legend to Fig. 1. The constant k_1 is expressed in micromoles of reduced coenzyme oxidized per minute per milligram of protein.

Additions to assay	k_1		K_1		K_2	
	DPNH	TPNH	DPNH	TPNH	DPNH	TPNH
			μM	μM	mM	mM
None	80	33	34	25	0.5	High
Chlorpromazine (200 μM)	75	22	34	13	0.04	High
Perphenazine (20 μM)	96		34		0.02	
ADP (100 μM)	162	73	40	46	1.0	High
ADP + chlorpromazine	162	73	40	46	0.4	High
GTP (100 μM)	1.4	0.8	5	5	0.6	High
GTP + chlorpromazine	1.1	0.4	10	5	0.4	High
GTP + perphenazine	0.8		10		2.0	

TABLE 2

Effects of purine nucleotides on modifier constants of chlorpromazine and perphenazine for glutamate dehydrogenase

Purine nucleotide	Chlorpromazine				Perphenazine	
	Dissociation		V/V'		Dissociation: DPNH	V/V': DPNH
	DPNH	TPNH	DPNH	TPNH		
	μM	μM			μM	μM
None	32, 16[a]	>200	6.0		5,[b] 20	50
ADP (100 μM)	>200	>200			1000, 6[c]	2[c]
ATP (100 μM)	95	>200	100	3.7	20	50
GTP (100 μM)	>200	36	4.3	3.0		

[a] The concentration of reduced coenzyme in all experiments was 100 μM except for this one, in which the concentration of DPNH was 300 μM. This change did not alter the value of V/V'.

[b] In this experiment a constant amount of glutamate dehydrogenase (4 or 8 μg/ml) was incubated with various concentrations of perphenazine (20–200 μM) for 10 min, after which 0.1 ml of the incubation mixture was added to the standard assay mixture (0.9 ml) to give a final volume of 1.0 ml. The dissociation constant refers to the concentration of perphenazine in the assay. The other experiments with perphenazine were performed by incubating perphenazine (20–200 μM), glutamate dehydrogenase (0.4–0.8 μg/ml), and the other constituents of the standard assay except DPNH for 5 min. Then DPNH was added and the solution was assayed.

[c] The dissociation constant refers to K_3 in Eq. 3, or the inhibition constant of the drug, in all experiments except these, in which the two values of the dissociation constants represent K_3 and K_4 in Eq. 4. Similarly, the ratio V/V' refers to the ratio V_1/V_2 in Eq. 3 except as noted here, where it refers to the ratio V_1/V_3 in Eq. 4.

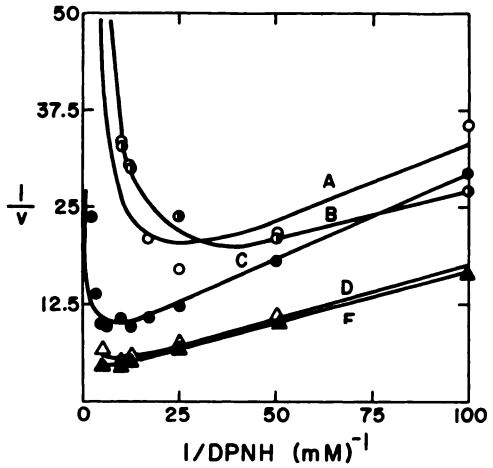


FIG. 1. *Double-reciprocal plot of velocity with respect to concentration of DPNH in the absence of any modifiers (curve C) and in the presence of 200 μM chlorpromazine (curve A), 20 μM perphenazine (curve B), 200 μM chlorpromazine plus 100 μM ADP (curve D), and 100 μM ADP alone (curve E)*

Experiments with perphenazine were performed by incubating perphenazine (200 μM) with 10 times more glutamate dehydrogenase than the concentration required to give the optimal rate in a standard assay (usually about 4–8 μg/ml) for 10 min in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25° Then 0.1 ml of this solution was added to the assay mixture to give a final volume of 1 ml. The curves were calculated with the use of Eq. 1. In all assays the concentration of α-ketoglutarate was 2 mM, and that of NH_4Cl was 50 mM.

proaches that of the undiluted sample (Fig. 4, curve *C*). These results can be interpreted as indicating that the enzyme-perphenazine complex (*E-P*) can slowly form a more inhibited complex (*EP**) as shown in Scheme 1. In this mechanism the complex *EP** is more inhibited, is formed more slowly, and is less reversible than *EP*. That is, the rate constant k_4 is smaller than k_3, and both are small with respect to k_1 and k_2.

$$E + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EP \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP^*$$

SCHEME 1

Previous results obtained with chlorpromazine are not consistent with the concept that this drug and ADP are bound to the same site on the enzyme, in spite of the fact that ADP markedly reduces inhibition by chlorpromazine (1). Similar results are obtained with perphenazine (Figs. 2 and 3). These results suggest that in the presence of ADP the drugs are bound to the enzyme but

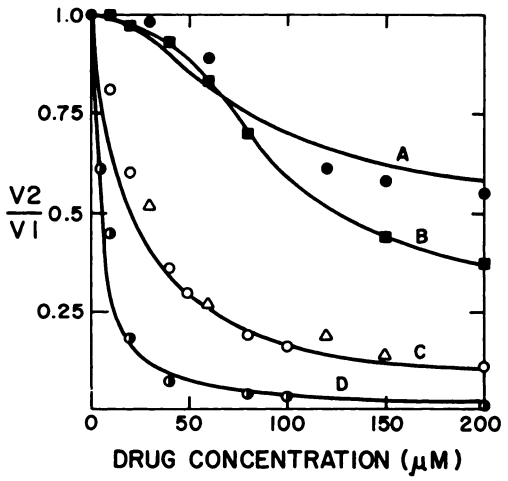


FIG. 2. *Plot of ratio of velocity in the presence (V_2) to that in the absence (V_1) of drug with respect to concentration of drug*

The drug is perphenazine in curves *A* and *C*, and haloperidol in curves *B* and *D*. These experiments were performed in the presence of either 100 μM DPNH (curves *A*, *C*, and *D*) or 1 mM DPN (curve *B*). Curve *A* shows results obtained in the presence of DPNH and 100 μM ADP, while curve *C* shows results obtained in presence of DPNH with (△) or without (○) 100 μM ATP. The reaction mixtures for curves *A*, *C*, and *D* also contained α-ketoglutarate (2 mM), ammonium chloride (50 mM), and glutamate dehydrogenase (0.4 μg/ml of assay mixture), and, for curve *B*, glutamate (10 mM) and glutamate dehydrogenase (4 μg/ml of assay mixture). Experiments with perphenazine (curves *A* and *C*) and haloperidol (curves *B* and *D*) were performed by incubating the drug (20–200 μM) with glutamate dehydrogenase and the other constituents of the standard assay except the coenzyme for 5 min in the case of perphenazine and for 2 min in the case of haloperidol. At the end of this time DPNH (*A*, *C*, and *D*) or DPN (*B*) was added to the mixture. Remaining experimental conditions are given in the legend to Fig. 1. Curves *A*, *C*, and *D* were calculated with the use of Eqs. 3 and 4 and the values given in Table 2. The points are experimental values. Although each ligand and coenzyme added altered the activity of glutamate dehydrogenase, the results shown in this figure are the ratios of velocity in the presence to that in the absence of drug, so that in absence of drug this value is unity in all the experiments shown.

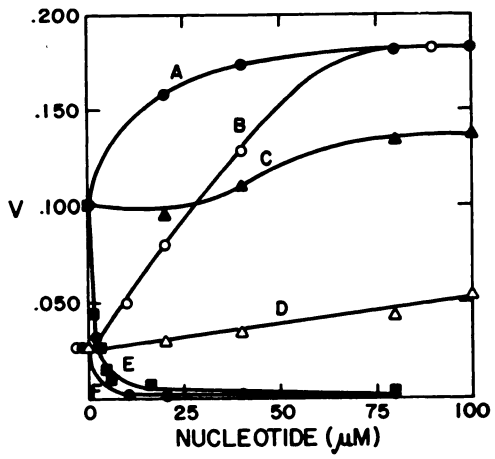


FIG. 3. *Plot of velocity with respect to concentration of purine nucleotides in the absence (curves A, C, and E) and presence (curves B, D, and F) of 20 μM perphenazine*

The nucleotides were ADP (curves *A* and *B*), ATP (curves *C* and *D*), and GTP (curves *E* and *F*). The reaction mixtures also contained DPNH (100 μM), α-ketoglutarate (2 mM), and ammonium chloride (50 mM). Experiments with perphenazine (curves *B*, *D*, and *F*) were performed by incubating 10 times more glutamate dehydrogenase than needed for an assay (4–8 μg/ml) with 200 μM perphenazine for 10 min in 0.025 M EDTA, pH 7.8, at 25°, after which 0.1-ml aliquots of the incubation mixture were added to the assay mixtures (0.9 ml) and assays were performed. Controls, which contained no perphenazine, were similarly incubated and diluted. The results of control experiments in the absence of both nucleotide and drug are shown on the left-hand ordinate. Incubations and assays were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

ADP prevents them from inhibiting enzyme activity. This conclusion is reinforced by the results shown in Table 3. Solutions of ADP, perphenazine, and enzyme were incubated for 10 min and then diluted 10-fold and assayed. If ADP and perphenazine are bound to the same site on the enzyme, ADP should prevent formation of the *EP* or *EP** complex in the incubated solutions, and there would be essentially no inhibition in the diluted samples. The activity in the diluted samples would be equal to that observed when 10-fold lower concentrations of enzyme, ADP, and perphenazine are incubated and assayed directly without dilution (Fig. 2, curve *A*). As shown in Table 3, this is not the case. When ADP, perphenazine, and enzyme were incubated together and then diluted, only the rapidly reversible activating effect of ADP was diminished, and the inhibitory effect of the slowly reversible *EP** complex remained. Furthermore, the

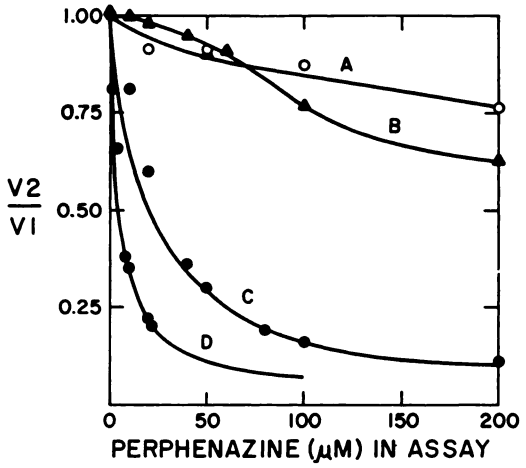


FIG. 4. *Plot of ratio of velocity in the presence (V_2) to that in the absence (V_1) of perphenazine with respect to concentration of perphenazine in the assay mixture*

These experiments were performed in the presence of 100 μM DPNH, with either 50 μM ammonium chloride (curves *A*, *C*, and *D*) or 1.0 mM DPN (curve *B*). The keto acid substrate was 0.9 mM pyruvate (curve *A*) or 2 mM α-ketoglutarate (curves *C* and *D*). Curve *B* shows the results obtained when 10 mM glutamate was the substrate. The glutamate dehydrogenase concentration in the assay mixture was 0.4–0.8 mg/ml (curve *A*), 4–8 μg/ml (curve *B*), or 0.4–0.8 μg/ml (curve *C*). Experiments with perphenazine shown in curve *D* were performed by incubating 10 times more glutamate dehydrogenase than necessary for an assay (4–8 μg/ml) with perphenazine (20–200 μM) for 10 min. At the end of this period 0.1-ml aliquots were added to the mixture (0.9 ml) containing the other constituents of the assay, and the mixture was assayed. Other experiments with perphenazine (curves *A*, *B*, and *C*) were performed by incubating the drug (20–200 μM) with glutamate dehydrogenase and other assay constituents except the coenzyme for 10 min, after which coenzyme (DPNH, curves *A*, *C*, and *D*; or DPN, curve *B*) was added and the mixture was assayed. Incubations and assays were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

TABLE 3

Effect of ADP on inhibition of glutamate dehydrogenase by perphenazine

In these experiments glutamate dehydrogenase (4–8 μg/ml) was incubated for 10 min either alone or with the concentrations of perphenazine and/or nucleotides shown. Then 0.1 ml of the incubated solution was added to 0.9 ml of the standard assay mixture, and this solution was assayed. Relative activity is the ratio of the activity of the solutions which contained perphenazine and/or nucleotides to that of a similar glutamate dehydrogenase solution incubated without these additions. Assays and incubations were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

Additions to incubation solution	Relative activity in diluted assay system
ADP (100 μM)	1.4
Perphenazine (200 μM)	0.22
ADP + perphenazine	0.40
ADP + DPNH (100 μM)	1.4
ADP + DPNH + perphenazine	0.40
DPNH + perphenazine	0.22

addition of DPNH did not alter these results (Table 3). Therefore ADP does not block formation of the *EP** complex, and ADP and perphenazine are not bound to the same site. High concentrations of ADP prevent bound perphenazine from inhibiting the enzyme.

In undiluted assays the effects of perphenazine in the presence of ADP are complicated (Fig. 2, curve *A*). A low concentra- of the drug produces essentially no effect whereas a higher concentration has a marked effect. Therefore curve *A* of Fig. 2 was calculated with the use of Eq. 4 and the values of the constants shown in Table 2.

Perphenazine has a slight inhibitory effect, and chlorpromazine has essentially none, on the rate of reduction of DPN (Figs. 4 and 5). It is believed that the substrate activation produced by DPN (more enzyme activity at high DPN concentration than is predicted by extrapolation from results obtained in the presence of low concentrations of DPN) results from binding of DPN to the ADP site (12). Binding of DPN, like ADP, to this site results in activation of the rate of DPN reduction at the active site. In this respect it is of interest that perphenazine in the presence of high concentrations of DPN has a sigmoid effect on velocity similar to its effect in the presence of ADP (Fig. 4, curve *B*); that is, a low concentration of perphenazine produces little inhibition whereas higher concentrations have a marked effect.

Neither chlorpromazine (1) nor perphenazine markedly inhibits alanine dehydrogenase activity in the presence of a high concentration of enzyme (Fig. 4, curve *A*).

Haloperidol. Although structurally different from chlorpromazine or perphenazine, this drug is the most potent inhibitor of those tested (Table 4 and Fig. 2). As with perphenazine, the maximal inhibitory effects of haloperidol are not immediately apparent, but require about 1 min of incubation to develop. This is shorter than the time required for maximal inhibition by perphenazine. Both haloperidol and perphenazine inhibit DPN reduction more than chlorpromazine (Fig. 2). Results obtained with a combination of haloperidol and chlorpromazine are consistent with the concept that both

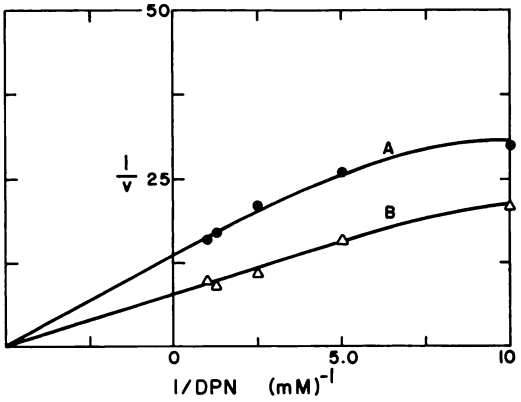


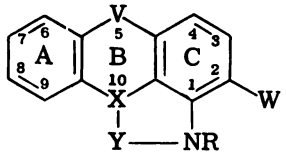
FIG. 5. *Double reciprocal plot of velocity with respect to concentration of DPN in the presence of 10 mM glutamate and in the presence (curve A) or absence (curve B) of 200 μM perphenazine*

Experiments with perphenazine were performed by incubating the drug (200 μM) with glutamate dehydrogenase and other constituents of the assay except for DPN. After incubation for 10 min, DPN was added to the mixtures in the concentrations indicated and the mixtures were assayed. All incubations and assays were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

TABLE 4

Structures and relative potencies of drugs tested

The terms K_3, V_1, and V_2 are the constants of Eq. 3 under standard assay conditions (see MATERIALS AND METHODS). Relative potency is the value of V_1/V_2K_3 for any given drug relative to promazine.



V	W	X	Y	R	Compound	K_3	V_1/V_2	Relative potency
						μM		
S	H	NH			Phenothiazine base	>400		
S	Cl	NH			2-Chlorphenothiazine base	>400		
S	H	N	—C—C—C—	$(CH_3)_2$	Promazine	80	3.0	1.0
S	OCH_3	N	—C—C—C—	$(CH_3)_2$	Methoxypromazine	54	2.0	1.0
S	CF_3	N	—C—C—C—	$(CH_3)_2$	Triflupromazine	80	6.0	2.0
S	Cl	N	—C—C—C—	$(CH_3)_2$	Chlorpromazine	32	6.0	5.0
SO	Cl	N	—C—C—C—	$(CH_3)_2$	Chlorpromazine sulfoxide	85	7.0	2.0
S	Cl	N	—C—C—C—	H; CH_3	Demonomethylchlorpromazine	72	11	4.0
S	Cl	N	—C—C—C—	$(H)_2$	Dedimethylchlorpromazine	200	11	1.5
S	Cl	N	—C—C—C—	$(CH_3)_3$	SKF 2680	30	3.0	3
S	Cl	N	—C—C—C—	$(C_2H_5)_2$	Chlorproethazine	25	6.0	6.0
S	Cl	N	—C—C—C—	$(—C—C—)_2N—CH_3$	Prochlorperazine	18	10	14.0
S	Cl	N	—C—C—C—	$(—C—C—)_2N—C—COH$	Perphenazine	20	50	63
S	CF_3	N	—C—C—C— | C C |	$(CH_3)_2$	Triflutrimeprazine	>400		
S	H	N	—C—C—	$(CH_3)_2$	Promethazine	155	3.0	0.5
S	H	N	—C—C—	$(CH_3)_2$	Fenethazine	290	4.0	0.3
S	H	N	—C—C—	$(C_2H_5)_2$	Diethazine	27	2.5	2.5
—C—C—	H	N	—C—C—C—	$(CH_3)_2$	Imipramine	80	4	1.3
—C—C—	H	N	—C—C—C—	H; CH_3	Desipramine	200	8	1.0

—C—C— C	H	>C=	=C—C—C—	$(CH_3)_2$	Amitriptyline	~400	20	1.3
—C—N—	H	>C=	=C—C—C—	$(CH_3)_2$	EX 10-029	70	1.5	0.5
—C—O—	H	>C=	=C—C—C—	$(CH_3)_2$	Doxepin	200	5	1.0
$—CH_3$	H	—C—	—O—C—C—	$(CH_3)_2$	Orphenadrine	135	3.5	0.7
					Haloperidol	4.0	100	625

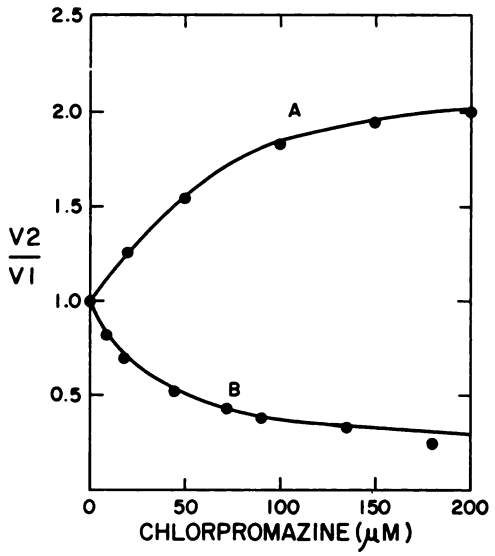


FIG. 6. *Plot of ratio of velocity in the presence (V_2) to that in the absence (V_1) of chlorpromazine with respect to concentration of chloropromazine in the absence (curve B) or presence (curve A) of 100 μM haloperidol*

The reaction mixtures also contained DPNH (100 μM), α-ketoglutarate (2 mM), and ammonium chloride (50 mM). Experiments with haloperidol were performed by incubating glutamate dehydrogenase with 100 μM haloperidol and other assay constituents except DPNH and chlorpromazine for 2 min, after which DPNH and chlorpromazine were added and the mixture was assayed. Other experimental conditions are given in the legend to Fig. 1.

drugs are bound to the same site on the enzyme (Fig. 6). Chlorpromazine (a poor inhibitor) is an activator in the presence of haloperidol (a more potent inhibitor), as would be expected if chlorpromazine displaced haloperidol from the enzyme.

Effects of other drugs. The effects of several other drugs on glutamate dehydrogenase are summarized in Table 4. It has previously been shown that ouabain, strophanthidin, phenobarbital, γ-aminobutyric acid, morphine, caffeine, quinine, and chlordiazepoxide have no significant effect on this enzyme (1). Dihydrochlorthiazide has also been found to have no inhibitory effect on glutamate dehydrogenase.

Spectroscopy and Fluorescence

Spectroscopy. Figure 7 shows the effect of chlorpromazine, perphenazine, and haloperidol on the absorption spectrum of glutamate dehydrogenase. These results represent the difference between the absorbance of glutamate dehydrogenase in the presence of drug and that of the drug alone. It can be seen that all three drugs increase the absorbance of glutamate dehydrogenase in the range between 260 and 280 mμ. In contrast, glutamate dehydrogenase has no significant effect on the absorption spectra of the drugs.

Fluorescence. Chlorpromazine absorbs light in the range between 300 and 340 mμ. When this drug is excited by light at 340 mμ, weak fluorescence with a peak between 460 and 470 mμ is observed (Fig. 8, curve *E*). Glutamate dehydrogenase has a typical protein emission spectrum with a peak at 340 mμ after excitation at 280 mμ (Fig. 8, curve *A*). If chlorpromazine is added to glutamate dehydrogenase and this solution is excited at 280 mμ, fluorescence at 340 mμ is decreased and the solution fluoresces between 450 and 460 mμ. Chlorpromazine alone does not fluoresce when excited at 280 mμ. These re-

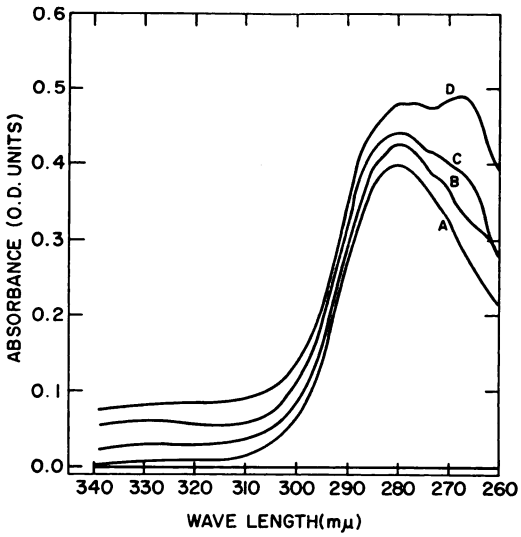


FIG. 7. *Absorption spectra of glutamate dehydrogenase (0.4 mg/ml) in the absence of drugs (curve A), or in the presence of 100 μM haloperidol (curve B), 100 μM chlorpromazine (curve C), or 100 μM perphenazine (curve D)*

Curves *B*, *C*, and *D* represent the difference spectra between the absorbance of glutamate dehydrogenase in the presence of the drug and that in the absence of enzyme. These experiments were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

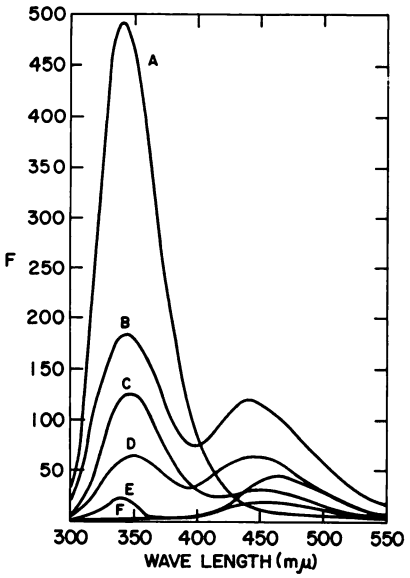


FIG. 8. *Fluorescence emission spectra of glutamate dehydrogenase (0.31 mg/ml), chlorpromazine (100 μM), and DPNH (33 μM)*

The exciting wavelengths were 280 mμ (curves *A–D* and *F*) and 340 mμ (curve *E*). The fluorescence spectra of glutamate dehydrogenase, chlorpromazine, and DPNH alone are shown in curves *A*, *E*, and *F*, respectively. The fluorescence spectra of a mixture of glutamate dehydrogenase plus DPNH and of chlorpromazine are shown in curves *B* and *C*, respectively. The fluorescence spectrum of the mixture of glutamate dehydrogenase, DPNH, and chlorpromazine is shown in curve *D*. These experiments were performed in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°.

sults are consistent with the concept that chlorpromazine bound to glutamate dehydrogenase can absorb the light emitted from the protein at 340 mμ and consequently it fluoresces at 450–460 mμ. Moreover, the slight increase chlorpromazine produces in enzyme absorbance at 280 mμ (Fig. 7) is negligible compared with the absorption of light emitted from the protein at 340 mμ. Therefore the effects of chlorpromazine on protein fluorescence are similar to the well-known effects of reduced pyridine nucleotides (23). Similarly, the effects of chlorpromazine on enzyme fluorescence can be used to estimate the dissociation constant of the enzyme-chlorpromazine complex (Fig. 9). Since chlorpromazine absorbs light at 280 mμ (the exciting wavelength in these experiments), the results shown in Fig. 9 are the difference between the effect of chlorpromazine on glutamate dehydrogenase and those on a tryptophan blank (see MATERIALS AND METHODS). Similar experiments can be performed with perphenazine. If it is assumed that there are six binding sites per enzymatically active monomer (mol wt 2.8×10^5) (15, 16) and the change in fluorescence is proportional to the amount of drug bound to the enzyme, the dissociation constants of chlorpromazine and perphenazine can be estimated to be 40 and 10 μM, respectively. In these estimates it is assumed that there are as many binding sites (six) for these drugs as there are sites for coenzyme and purine nucleotides (13, 14, 18), and that the change in fluorescence is related to the concentration of free or unbound drug in a manner similar to the change in velocity as expressed by Eq. 3. The curves shown in Fig. 9 have been calculated with these assumptions. These values of the dissociation constants are similar to the inhibition constants (K_3) of these drugs.

The addition of both chlorpromazine and DPNH to the enzyme produces more quenching of enzyme tryptophan fluorescence (340 mμ) than the addition of either DPNH or chlorpromazine alone (Fig. 8). Chlorpromazine markedly decreases the fluorescence of the DPNH-enzyme mixture at 460 mμ, but the addition of DPNH only slightly enhances the fluorescence of the mixture of chlorpromazine and enzyme at this wavelength. Consequently, in spite of the enhanced quenching at 340 mμ, it seems unlikely that chlorpromazine enhances binding of the more fluorescent (at 460 mμ) DPNH. What seems more probable is that chlorpromazine, in the presence of DPNH, produces a conformational change in the enzyme which results in a decreased emission of enzyme tryptophan at 340 mμ and consequently a decreased fluorescence of enzyme-bound DPNH and chlorpromazine at 460 mμ.

Effect of Pyridoxal Phosphate

Pyridoxal phosphate can form a Schiff base with one lysine group on each of the six peptide chains of the enzymatically active glutamate dehydrogenase monomer.

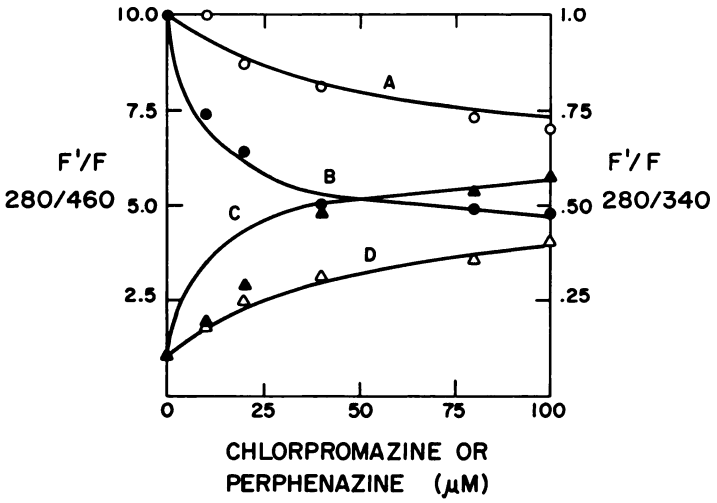


FIG. 9. *Plot of ratio of fluorescence in the presence of a drug (F') to that in its absence (F) as a function of drug concentration*

Results obtained with chlorpromazine are shown in curves *A* and *D*, and with perphenazine, in curves *B* and *C*. In all experiments the solutions were excited at 280 mμ. The left-hand ordinate gives the value of this ratio when fluorescence was read at 460 mμ (curves *C* and *D*). The right-hand ordinate gives the value of this ratio when fluorescence was read at 340 mμ (curves *A* and *B*). The curves were calculated by assuming that there are six drug-binding sites per active enzyme monomer and that the dissociation constants of chlorpromazine and perphenazine are 40 and 10 μM, respectively (see the text). Experiments were performed in 0.025 M sodium arsenate–0.1 M EDTA, pH 7.8, at 25°.

This results in a marked decrease in both alanine and glutamate dehydrogenase activity (24). In the experiments shown in Table 5, the reaction between enzyme and pyridoxal phosphate is slow and reaches completion after 1 hr if the concentration of pyridoxal phosphate is saturating (between 0.1 and 0.3 mM) and the concentration of enzyme is 0.2 mg/ml. At the end of 1–2 hr of incubation the enzyme retains about 8% of its original activity. Table 5 shows the effects of coenzyme, purine nucleotides, and some drugs on enzyme inactivation in the presence of saturating concentrations of pyridoxal phosphate. In these experiments rather high concentrations of enzyme were present in the incubation system, and therefore, after dilution, only a small amount of the incubated nucleotide or drug was present in the assay system. This was the case even with perphenazine. Control experiments showed that perphenazine has only a slight inhibitory effect if the original incubation solution containing 200 μM perphenazine is diluted 100-fold.

The results shown in Table 5 demonstrate that the combination of reduced pyridine nucleotide with GTP but not with ADP protects the enzyme. The combination of DPN with either ADP or GTP does not protect. None of the coenzymes or purine nucleotides alone gave significant protection. Treatment of the enzyme with pyridoxal phosphate did not alter the apparent Michaelis constant of any of the coenzymes or substrates, but the allosteric modification afforded by purine nucleotides was markedly decreased (Fig. 10). Therefore the reactions of the enzyme with pyridoxal phosphate and acetic anhydride are similar (25). In both cases the results can be interpreted on the assumption that one lysine group is quite reactive with both reagents. This group is not part of either the active or allosteric site, but is essential for optimal enzyme activity and allostery. Protection by GTP but not by ADP plus reduced coenzyme is not the result of direct masking of this lysine group by either GTP or DPNH; instead, the conformational change produced by GTP and

TABLE 5

Effects of nucleotides, coenzymes, and drugs on reaction between glutamate dehydrogenase and pyridoxal phosphate

Incubations were conducted for 1 hr in 0.025 M sodium arsenate–0.1 mM EDTA, pH 7.8, at 25°, with 0.3 mM pyridoxal phosphate and glutamate dehydrogenase (0.2 mg/ml). The concentrations of nucleotides added were 0.1 mM for DPNH, GTP, and ADP and 1.0 mM for DPN. The concentrations of drugs added to the incubation mixture were 0.2 mM for haloperidol and chlorpromazine and 0.3 mM for perphenazine. After incubation, the samples were diluted with arsenate buffer and assayed by the standard procedure. Residual activity is the ratio of activity of the treated sample to that of a control solution containing the same concentration of glutamate dehydrogenase incubated in the absence of pyridoxal phosphate. During these incubations no significant amount of enzyme activity was lost from control solutions.

Additions to incubation mixture	Residual activity
	%
None	8
Chlorpromazine	4
DPNH	30
DPNH + chlorpromazine	21
DPNH + perphenazine	17
DPNH + haloperidol	14
GTP	12
GTP + DPNH	76
GTP + DPNH + chlorpromazine	63
TPNH	36
TPNH + chlorpromazine	26
TPNH + GTP	60
TPNH + GTP + chlorpromazine	54
DPNH + ADP	22
DPNH + ADP + chlorpromazine	18
GTP + DPN	12
ADP + DPN	12

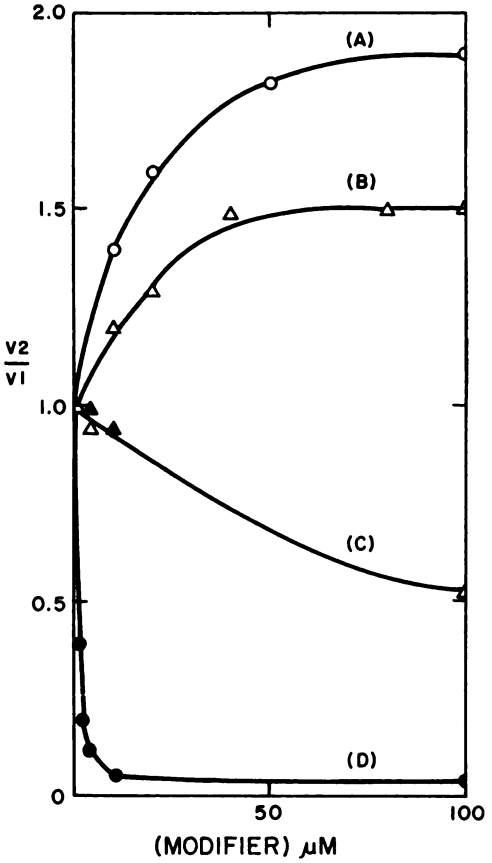


FIG. 10. *Plot of ratio of velocity in the presence of a modifier (V_2) to that in the absence of a modifier (V_1) as a function of modifier concentration*

ADP was the modifier for curves *A* and *B*, and GTP for curves *C* and *D*. Curves *B* and *C* show results obtained with the pyridoxal phosphate-treated enzyme (8% residual activity), and curves *A* and *D* show the results obtained with the native enzyme. Assays were performed in the standard system. Remaining experimental conditions are given in the legend to Fig. 1.

DPNH results in inhibition of enzyme activity and burying of this group so that it is no longer accessible to attack by pyridoxal phosphate or acetic anhydride.

As shown in Table 5, chlorpromazine, perphenazine, and haloperidol enhance the inactivation by pyridoxal phosphate. Therefore the lysine group is apparently not part of the drug-binding site. If the enzyme is treated with pyridoxal phosphate, chlorpromazine (in concentrations as high as 200 μM) has no effect on enzyme activity. The functional integrity of this lysine group is therefore also necessary for inhibition by chlorpromazine.

Ultracentrifugation

In the presence of DPNH, perphenazine, like chlorpromazine, decreases the sedimentation coefficient of glutamate dehydrogenase 1.4-fold (1).

DISCUSSION

The results presented above show that several drugs known to affect behavior are

bound to and inhibit glutamate dehydrognease. It has been shown previously that chlorpromazine is not competitive with respect to any of the substrates or coenzymes of the reaction. Since chlorpromazine and perphenazine are inhibitors in the presence of saturating concentrations of GTP, these drugs are obviously not competitive with respect to this purine nucleotide (1). Neither drug has a significant effect in the presence of ADP. It was previously concluded that if ADP and chlorpromazine were competitive a higher degree of inhibition would be produced by chlorpromazine than that actually observed (1). This is perhaps a tenuous conclusion. However, if ADP and perphenazine are incubated with glutamate dehydrogenase and this solution is then diluted and assayed, the freely reversible activating effect of ADP, but not the inhibitory effect of perphenazine, is lost. Therefore ADP does not prevent binding of perphenazine to the enzyme.

The drugs do not protect the enzyme from reacting with pyridoxal phosphate. Therefore the lysine group on the enzyme which reacts with pyridoxal phosphate and is important for full allostery and enzyme activity is apparently not part of the drug-binding site. After this group reacts with pyridoxal phosphate, even high concentrations of chlorpromazine no longer inhibit the enzyme. Therefore, if the lysine group is treated with pyridoxal phosphate, chlorpromazine can no longer inhibit the enzyme.

While haloperidol has a considerably different structure from those of chlorpromazine or isosteres of phenothiazine, its effect on glutamate dehydrogenase is quite similar. Experiments performed with chlorpromazine and haloperidol are consistent with the concept that both drugs are bound to the same enzyme site.

The results are consistent with the concept that certain drugs which affect behavior are bound to a unique site on glutamate dehydrogenase. Binding of drugs to this "drug site" produces inhibition of enzyme activity, a decrease in the sedimentation coefficient (or dissociation) of the enzyme, a decrease in the fluorescence, and an increase in the absorbance of the enzyme. These results suggest that inhibition by these drugs is secondary to an induced change in the conformation of the enzyme.

In general the drugs are more inhibitory if the enzyme is already in a rather inactive conformation. Perhaps inhibition of the enzyme, whether by high concentrations of DPNH or GTP, facilitates further drug-induced inhibition. The drugs produce less inhibition, when the enzyme is activated by ADP or DPN. In this case, after a certain fraction of drug-binding sites is occupied, the enzyme acquires a greater affinity for drugs, and binding results in more inhibition. In the presence of these drugs considerably more ADP must be added to produce full activation of enzyme activity (Fig. 3, curve *B*). Again these results can be interpreted as indicating that although ADP and perphenazine are not competitive, each decreases the affinity of the enzyme for the other. Perphenazine cannot prevent full activation by ADP, but ADP can abolish the maximal, potential inhibitory effect of perphenazine. ATP, which is a weaker activator than ADP, is much less capable of preventing inhibition by these drugs.

K_s, the dissociation constant for promazine and other drugs at the drug site on the enzyme in the presence of DPNH, can be used to correlate the effects of modification of the chemical structure of promazine with binding to the drug site. It can be seen from the results in Table 4 that while promazine is an inhibitor of glutamate dehydrogenase, phenothiazine is not. Therefore the side chain on N-10 is required for inhibition. A 3-carbon side chain between N-10 and the terminal amino nitrogen results in optimal binding. In general, shortening of the chain to 2 carbon atoms markedly decreases binding except for diethazine, which has a bulky substituent on the terminal nitrogen. Bulky substituents on the terminal nitrogen result in increased binding to the enzyme, as in the case of diethazine, chlorproethazine, prochlorperazine, perphenazine, and haloperidol. Furthermore, some drugs with these bulkier substituents, such as perphenazine, haloperidol, and prochlorperazine, produce more inhibition if they have previously

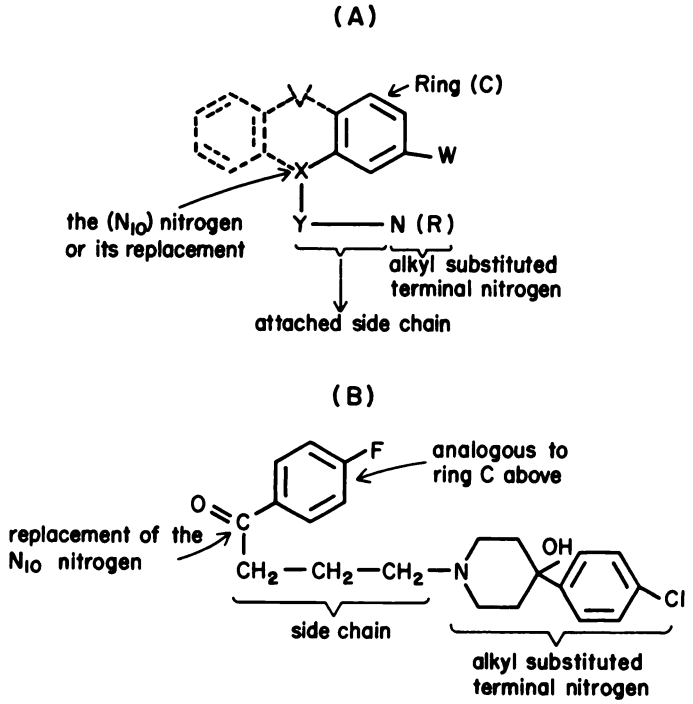
been incubated with the enzyme. This suggests that a slow, measurable time interval is required for reorientation of the bulky groups in the enzyme-drug complex. Compounds containing smaller substituents on the terminal amino group, such as chlorpromazine, exert maximal inhibition immediately. Mono- and didemethylation of the terminal amino groups result in a progressive decrease in binding, as seen with demonomethylchlorpromazine, dedimethylchlorpromazine, and demethylimipramine. Addition of a methyl group to the terminal amino nitrogen to give a quaternary amino group, as in SKF 2680, does not affect binding; SKF 2680 has a K_3 value similar to that of chlorpromazine. Branching of the side chain results in marked reduction in binding of drugs to the enzyme, as is the case for promethazine.

Small electron-withdrawing substituents on C-2 enhance binding, as in the case of methoxypromazine and chlorpromazine. Larger electron-withdrawing groups seem to have no effect, as seen with the CF_3 group on triflupromazine. Replacing the sulfur in ring B with an ethylene bridge, as with imipramine, does not affect binding, and sulfoxidation of the sulfur slightly decreases binding, as in chlorpromazine sulfoxide. This suggests that this region of the molecule does not markedly affect binding. Replacement of N-10 by a methylene group may or may not decrease binding, depending on the other substituents present on the molecule, as in the case of amitriptyline, doxepin, orphenadrine, and EX 10-029. The large difference in binding between amitriptyline and imipramine suggests that substituting a methylene group for N-10 decreases binding. However, some replacements for N-10, as in EX 10-029, result in binding essentially as strong as that of imipramine. Ring B does not seem to be necessary for binding, as seen with haloperidol.

It can be inferred from these results with isosteres of phenotriazine and haloperidol that the parts of the drug molecule which are most important for interaction with the binding site on the enzyme are the phenyl ring (ring C in the case of the substituted phenothiazine, and the phenyl ring adjacent to the carbonyl group in haloperidol), N-10 (or its proper replacement) with its attached side chain, and the alkyl-substituted terminal nitrogen. This is similar to other proposed models (26, 27) for the pharmacologically active parts of the substituted phenothiazine molecule. The basic structure important for binding to glutamate dehydrogenase is shown in Scheme 2A. Although haloperidol has a different structural formula from that of substituted phenothiazine, it can conceivably assume a conformation resembling the basic structure necessary for binding (Scheme 2B).

A correlation of these results with known effects of the drugs *in vivo*, based only on the kinetic constant K_3, is very difficult. While K_3 is apparently an estimate of the affinity of the drug for the drug site on the enzyme, this constant does not reflect the degree of inhibition produced by the drug after binding. The ratio V_1/V_2 is a measure of the magnitude of inhibition, but it does not take into account the concentration of the drug required for inhibition. In general, however, correlations can be made between the effects of these drugs on glutamate dehydrogenase and their antipsychotic activity *in vivo* if all three constants are considered and the effective potency *in vitro* is defined as the ratio V_1/V_2K_3. To simplify these correlations further, the effective potencies of the various drugs are expressed as multiples relative to the effective potency of promazine, which is arbitrarily set equal to unity. Promazine has been used previously as a basis for comparing tranquilizer activities (28). The phenothiazine nucleus alone neither inhibits glutamate dehydrogenase nor has antipsychotic activity, but promazine exhibits both activities (Table 4) (29, 30). Both activities are enhanced by substitution of electron-withdrawing groups on position 2 of ring C, as in chlorpromazine and triflupromazine (29, 31), but not methoxypromazine (32–34). Substitution of an *N*-methylpiperazinylpropyl group at N-10 (prochloperazine), or of an *N*-hydroxyethylpiperazinyl group (perphenazine),

SCHEME II



markedly enhances both activities (Table 4) (31, 35). A progressive decrease in both activities results from progressive demethylation of the terminal nitrogen to give demonomethylchlorpromazine and dedimethylchlorpromazine, respectively (36, 37). Demonomethylchlorpromazine is only slightly less active both as an inhibitor of glutamate dehydrogenase and in antipsychotic potency than its parent compound, chlorpromazine (Table 4) (36, 37). Addition of an extra methyl group to the terminal nitrogen of the side chain (e.g., SKF 2680) results in only a 3-fold increase in relative effective potency with respect to glutamate dehydrogenase. SKF 2680 is thought to be devoid of antipsychotic properties because of its inability to cross the blood-brain barrier.

Shortening of the chain length to 2 carbons (fenethazine) or the presence of a branched chain (promethazine and triflutrimeprazine) reduces both relative effective potency with respect to glutamate dehydrogenase and antipsychotic activity (31). Sulfoxidation of chlorpromazine results in a marked decrease in both activities (30, 38, 39) (Table 4). In addition, structural modifications which enhance antidepressant, antiparkinsonian, or antihistaminic activity, as in demethylimipramine, orphenadrine, and triflutrimeprazine, result in reduction of the inhibitory effects of these compounds on glutamate dehydrogenase (Table 4) (31, 37). Chlorproethazine and, to some extent, diethazine are exceptions.

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