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Effects of Tranquilizers on the Glutamate Dehydrogenase–Glutamate-Oxalacetate Transaminase Complex

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SUMMARY

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Perphenazine and haloperidol have been shown to be potent inhibitors of the reactions of keto or amino acids with glutamate dehydrogenase. These drugs do not significantly inhibit amino acid dehydrogenase reactions catalyzed by the complex formed between glutamate dehydrogenase and glutamate-oxalacetate transaminase, even though, in the presence of transaminase, they can still bind to glutamate dehydrogenase. Apparently, when these drugs are bound to glutamate dehydrogenase in the enzyme-enzyme complex, they cannot alter the conformation of this enzyme in the same manner that results in inhibition of the free enzyme. The transaminase-glutamate dehydrogenase complex catalyzes many amino acid dehydrogenase reactions with substrates such as tyrosine and aspartate. Since the enzyme-enzyme complex is not inhibited by these drugs, the amounts of transaminase and glutamate dehydrogenase in mitochondria are an important determinant of the degree of inhibition produced by these tranquilizers. If the levels of both enzymes are low, amino acid dehydrogenation would not be catalyzed by the complex, and perphenazine and haloperidol would be quite potent inhibitors. If the levels of both enzymes are high, the amino acid dehydrogenase reaction would be catalyzed mainly by the enzyme-enzyme complex, and these drugs would not be significant inhibitors.

INTRODUCTION

It has been recently found that some tranquilizers, such as perphenazine, but not antidepressants, of a structure resembling desipramine, are potent inhibitors of glutamate synthesis through glutamate dehydrogenase [L-glutamate:NAD(P) oxidoreductase, EC 1.4.1.3] (1). Haloperidol, also a tranquilizer, was found to be a very potent inhibitor of glutamate dehydrogenase, but promethazine, which is structurally similar to perphenazine but lacks tranquilizer potency, does not inhibit glutamate dehydrogenase (1). In general there was a correlation between the inhibitory effect of these drugs

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on glutamate dehydrogenase and their therapeutic action. Several other experiments suggested that the enzyme-inhibitory effects of these drugs could be pharmacologically significant: (a) glutamate (2–5) and glutamate dehydrogenase (6) have important neurochemical functions, and glutamate dehydrogenase is present in high levels in brain (7–9); (b) the inhibition constant of tranquilizers such as haloperidol is quite low (1); (c) these drugs do not inhibit other enzymes of glutamate metabolism, including glutamate-oxalacetate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.11) and glutamine synthetase (9); (d) haloperidol has been tested *in vivo* and has been found to lower brain levels of glutamate (10); and (e) these drugs are not simple competitive inhibitors of glutamate dehydrogenase but apparently are bound to a unique site on the enzyme (1, 9).

Glutamate dehydrogenase in bovine brain and liver (7, 9) is kinetically and electrophoretically identical and is affected similarly by the tranquilizers. Therefore these drugs probably do not inhibit the brain enzyme selectively on the basis of its unique properties. Possibly organ specificity is determined by the microenvironment. For example, high DPNH:DPN, ATP:ADP, DPNH:TPNH, or GTP:ADP ratios favor inhibition of glutamate dehydrogenase by some tranquilizers *in vitro* (1, 9). Alterations of these ratios in mitochondria *in vivo* might result in inhibition of the brain rather than the liver enzyme.

Another factor which could alter the effects of these drugs on glutamate dehydrogenase in different organs may be the level of other mitochondrial proteins, such as glutamate-oxalacetate transaminase. This is because in the presence of reduced pyridine nucleotides and NH_4^+ glutamate dehydrogenase can reversibly catalyze the conversion of this enzyme from the pyridoxal to the pyridoxamine phosphate form (11–14). Thus, in the presence of some amino acids, oxidized pyridine nucleotides, and both enzymes, certain amino acid dehydrogenase reactions may be catalyzed by the enzyme-enzyme system but not by glutamate dehydrogenase alone. For example, tyrosine does not react with glutamate dehydrogenase alone, but it does participate in a transaminase half-reaction with the pyridoxal phosphate form of the transaminase (14–16) and consequently reacts with DPN to produce equimolar amounts of DPNH, NH_4^+, and hydroxyphenylpyruvate in the enzyme-enzyme system (14).

In reactions between glutamate dehydrogenase and transaminase a complex is apparently formed between the two enzymes, and the glutamate dehydrogenase in this complex reacts with transaminase-bound pyridoxal or pyridoxamine phosphate. The reaction is not mediated by keto or amino acids associated with either enzyme, and the reactive group is tightly bound to the enzyme (11, 13, 14). Pyridoxal or pyridoxamine phosphate is required for the reaction (11, 13, 14), and during the reaction these coenzymes are not transferred from transaminase to glutamate dehydrogenase (11). Moreover, the two enzymes must be in direct contact to react (13). Finally, in the presence of glutamate dehydrogenase, the molecular weight of the transaminase is increased (as estimated by Sephadex gel filtration, see ref. 14).

The effects of perphenazine and haloperidol on reactions catalyzed by the enzyme-enzyme complex are the subject of this paper. These two drugs were used because they are the most potent tranquilizers inhibiting glutamate dehydrogenase (1).

In this study crystalline liver rather than brain mitochondrial enzymes were used, since the former are easier to prepare and crystallize (9, 17, 18). Because no differences have been found between bovine liver and brain mitochondrial glutamate-oxalacetate transaminase and glutamate dehydrogenase (7, 9), it appears valid to extrapolate from the liver to the brain enzyme. A few experiments were performed with pig heart glutamate-oxalacetate transaminase; again no differences were found between the heart and brain preparations (16).

MATERIALS AND METHODS

Enzymes and reagents. Bovine liver mitochondrial glutamate dehydrogenase and glutamate oxalacetate-transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.11) were prepared and crystallized by

methods described previously (17–19). Cytoplasmic pig heart glutamate-oxalacetate transaminase was purchased from Boehringer/Mannheim Corporation. The pyruvate used was triply distilled. Other substrates, coenzymes, nucleotides, and enzymes were obtained from Sigma Chemical Company. Stock solutions of all reagents were adjusted to the pH of the assay system and prepared as sodium salts. Enzymes were dialyzed for 20 hr prior to use in these experiments against 2 liters (two volume changes) of 0.025 M sodium arsenate and 0.1 mM EDTA, pH 7.8, at 4°. [4-^{3}H]DPN was obtained from New England Nuclear Corporation. The transaminase apoenzyme was prepared according to previously described methods (20, 21).

Measurement of protein concentration. Enzyme concentration was measured spectrophotometrically at 280 or 360 nm with the use of previously published extinction coefficients (12, 18–23). Enzyme concentration is expressed in units of moles of enzyme peptide chain per liter, using 5×10^4 and 5.6×10^4, respectively, as the molecular weights of transaminase and glutamate dehydrogenase peptide chains (24, 25).

Initial velocity measurements. Enzyme assays were carried out in 0.025 M sodium arsenate with 0.1 mM EDTA, pH 7.8, at 25°. Reactions were followed spectrophotometrically at 340 or 360 nm with a Gilford model 2000 multiple sample absorbance recorder attached to a Beckman DU monochromator. Previously described precautions were used to measure initial velocity in the presence of drugs which absorb light at 340 nm (1, 9).

Drugs. The drugs were gifts from Schering (perphenazine) and McNeil Laboratories (haloperidol, Haldol). Both were obtained in the free base form and dissolved in 0.01 M HCl. Solutions of these drugs were prepared fresh daily and protected from exposure to light. The drug solutions were found to be stable during the time required to perform the experiments. The concentrations of drugs used in assays were low so that additions to the enzyme assay mixture did not alter the pH.

Synthesis of radioactive compounds. Tritiated DPNH and alanine were synthesized according to previously described methods (13). Radioactivity assays were performed as described previously (13).

Amino acid analysis. Amino acid analyses were performed with a Beckman model 120C amino acid analyzer, using previously described methods (13).

Identification of products. Measurements of oxidized pyridine nucleotide and of tritiated water were performed by previously described methods (13).

RESULTS

Enzyme-enzyme complex. Reductive amination of the pyridoxal phosphate form (GT-PLP) of the transaminase to the pyridoxamine form of the enzyme (GT-PMP) by TPNH (or DPNH) plus NH_4^+ (reaction 2) and the tyrosine dehydrogenase reaction (in the absence of α-ketoglutarate or glutamate) (reactions 1 and 2) both require the glutamate dehydrogenase–glutamate-oxalacetate transaminase enzyme-enzyme complex. Since glutamate dehydrogenase does not react with tyrosine directly, this latter reaction takes place as

$$\text{Amino acid} + \text{GT-PLP} \rightleftharpoons \text{keto Acid} + \text{GT-PMP} \quad (1)$$

$$H_2O + \text{DPN (or TPN)} + \text{GT-PMP} \rightleftharpoons \text{DPNH (or TPNH)} + NH_4^+ + \text{GT-PLP} \quad (2)$$

where glutamate dehydrogenase catalyzes reaction 2. Similarly, the reversible aspartate dehydrogenase reaction is slower in the presence of glutamate dehydrogenase alone than in the presence of both enzymes (12, 14). These reactions, which are catalyzed mainly or exclusively by the enzyme-enzyme complex, are not inhibited by haloperidol or perphenazine at concentrations sufficiently high to inhibit reactions catalyzed by glutamate dehydrogenase alone (Fig. 1). Therefore these two tranquilizers, which are potent inhibitors of glutamate dehydrogenase in the absence of transaminase (1), do not significantly inhibit reactions catalyzed by the enzyme-enzyme complex.

Enzyme-enzyme vs. enzyme system. High levels of glutamate dehydrogenase are required to catalyze the reductive amination of pyruvate or oxalacetate in the absence of

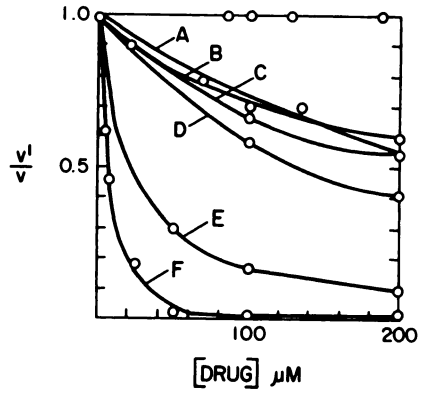

FIG. 1. *Plot of ratio of velocity in absence and presence of drug with respect to drug concentration*

When the reaction was catalyzed by the enzyme-enzyme complex, perphenazine and haloperidol had no effect at the levels of drug used, and thus the ratio is equal to 1. These assays were (a) the tyrosine dehydrogenase reaction in the presence of perphenazine, mitochondrial glutamate-oxalacetate transaminase (1.5 μM), glutamate dehydrogenase (0.24 mg/ml), DPN (1.0 mM), and tyrosine (4.5 mM); (b) the aspartate dehydrogenase reaction in the presence of perphenazine, mitochondrial glutamate-oxalacetate transaminase (0.5 μM), TPN (1.0 mM), aspartate (10 mM), and glutamate dehydrogenase (0.5 mg/ml); (c) the reductive amination of oxalacetate in the presence of perphenazine, mitochondrial glutamate-oxalacetate transaminase (0.6 μM), glutamate dehydrogenase (0.38 mg/ml), TPNH (100 μM), NH_4Cl (50 mM), and oxalacetate (100 μM); (d) the reductive amination of transaminase-bound pyridoxal phosphate (PLP) in the presence of perphenazine, cytoplasmic glutamate-oxalacetate transaminase (20 μM), DPNH (20 μM), NH_4Cl (50 mM), and glutamate dehydrogenase (1.0 mg/ml); and (e) the reductive amination of pyruvate in the presence of haloperidol or perphenazine and pyruvate (1.0 mM), NH_4Cl (50 mM), DPNH or TPNH (20 μM), glutamate dehydrogenase (0.5–1 mg/ml), and cytoplasmic glutamate-oxalacetate transaminase (20 μM). In the above assay the tyrosine dehydrogenase reaction, the reductive amination of low levels (100 μM) of oxalacetate, and the reductive amination of transaminase-bound pyridoxal phosphate require both enzymes (11, 12, 14). In the other assays the reaction is catalyzed predominantly by the enzyme-enzyme complex (see the text and refs. 11, 12, and 14). The effects of haloperidol or perphenazine on reactions catalyzed by glutamate dehydrogenase in the absence of the transaminase holoenzyme are shown in curves *A–F*: curve *A*, reductive amination of α-ketoglutarate in the presence of perphenazine, α-ketoglutarate (100 μM), TPNH (100 μM), NH_4Cl (50 mM), and glutamate dehydrogenase (1 μg/ml); *B*, reductive amination of pyruvate in the presence of haloperidol, pyruvate (1.0 mM), NH_4Cl (50 mM), glutamate dehydrogenase (0.5 mg/ml), and TPNH (100 μM); *C*, reductive amination of pyruvate in the presence of perphenazine, pyruvate (1.0 mM), NH_4Cl (50 mM), DPNH (20 μM), and glutamate dehydrogenase (1.0 mg/ml); *D*, the reductive amination of oxalacetate in the presence of perphenazine, oxalacetate (500 μM), TPNH (100 μM), NH_4Cl (50 mM), and glutamate dehydrogenase (0.5 mg/ml), with or without 20 μM cytoplasmic glutamate-oxalacetate transaminase *apoenzyme*; *E*, reductive amination of α-ketoglutarate in the presence of α-ketoglutarate (2.0 mM), NH_4Cl (50 mM), DPNH (100 μM), glutamate dehydrogenase (0.4 μg/ml), and perphenazine; *F*, same as curve *E* but with perphenazine replaced by haloperidol. All constituents were incubated at 25° for 5 min, after which the glutamate dehydrogenase-catalyzed reactions were initiated by adding the pyridine nucleotide coenzyme. These experiments were performed in 0.025 M sodium arsenate and 0.1 mM EDTA, pH 7.8, at 25°.

transaminase (3). However, as transaminase is added, this enzyme displaces the keto acid from glutamate dehydrogenase so that the reaction becomes catalyzed by the enzyme-enzyme complex (reactions 1 and 2). That is, as the level of transaminase is increased, the pyridoxal phosphate form of this enzyme, rather than the keto acid, reacts with glutamate dehydrogenase. This has been inferred from kinetic experiments (12) and is confirmed by the following direct experiments.

In these experiments 2-ml solutions of tritiated DPNH (with tritium on the B side of the pyridine ring) (100 μM; specific activity, 300 to 400 cpm/nmole), NH_4Cl (50 mM), pyruvate (1.0 mM), and glutamate dehydrogenase (0.13 mg/ml) were incubated for 75 min in the presence or absence of cytoplasmic transaminase (1.0 mg/ml). At the end of the incubation the concentration of DPN was measured and the solutions were deproteinized and applied to the amino acid analyzer. Alternatively, the reaction mixture was chromatographed on a column of DEAE-Sephadex as previously described (13), and the fractions containing tritium and/or alanine were then chromatographed in the amino acid analyzer. Identical results

were obtained with either method. Equal amounts of DPN and alanine were produced in the absence of transaminase, while slightly more DPN than alanine (100 vs. 70 μM) was produced in its presence. In the latter experiment the transaminase remained in the pyridoxal form. In the absence of transaminase all the tritium was eluted from the analyzer with alanine. In the presence of transaminase essentially all the tritium was eluted as tritiated water. These results are consistent with the concept that ammonia is incorporated by the transaminase and not by glutamate dehydrogenase and that the keto acid reacts with the amine form of the transaminase. It seems unlikely that all the alanine in the experiments above was produced via the glutamate dehydrogenase reaction and that the alanine exchanged tritium with water by reacting with the pyridoxal form of the transaminase. In control experiments [α-^{3}H]alanine, unlike glutamate, could not exchange all its tritium with water by reacting with glutamate-oxalacetate transaminase (13). When the glutamate-pyruvate transaminase is incubated with [α-^{3}H]alanine, essentially all the tritium is transferred to water (13).

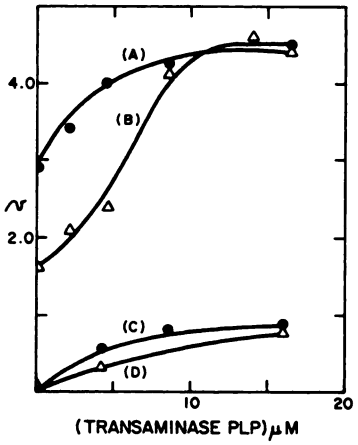

FIG. 2. *Plot of velocity (micromoles per liter per minute) of DPN production with respect to concentration of cytoplasmic glutamate-oxalacetate transaminase-bound pyridoxal phosphate (PLP) in the presence (curves A and B) and absence (curves C and D) of 1.0 mM pyruvate and the presence (curves B and D) and absence (curves A and C) of 200 μM perphenazine*

The concentration of glutamate dehydrogenase was 1.0 mg/ml; DPNH, 20 μM; and ammonium chloride, 50 mM. All constituents of the assay except DPNH were incubated at 25° for 5 min. At the end of this time DPNH was added. The experiments were performed in 0.025 M sodium arsenate and 0.1 mM EDTA, pH 7.8, at 25°.

Although perphenazine and haloperidol inhibit the reductive amination of pyruvate or oxalacetate by glutamate dehydrogenase alone (Figs. 2–4), they have no significant effect when the level of transaminase is sufficiently high for these reactions to be mediated by the enzyme-enzyme complex (Figs. 2–4). The transaminase apoenzyme, which does not react with glutamate dehydrogenase, did not alter the effect of these drugs on these reactions (Fig. 1).

DPNH oxidase activity. Cytoplasmic glutamate-oxalacetate transaminase can oxidixe DPNH (but not TPNH) in the presence of NH_4^+. This reaction (DPNH oxidation) can take place with the transaminase apoenzyme or pyridoxamine phosphate form as well as the pyridoxal phosphate form of the enzyme. For these and other reasons it has been concluded that in this reaction a group,

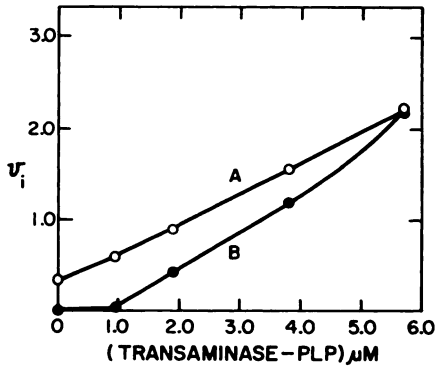

FIG. 3. *Plot of velocity (micromoles per liter per minute) of TPN production with respect to concentration of mitochondrial glutamate-oxalacetate transaminase-bound pyridoxal phosphate (PLP) in the absence (curve A) and presence (curve B) of 100 μM perphenazine*

Additional experimental conditions were: TPNH, 100 μM; ammonium chloride, 50 mM; oxalacetate, 100 μM; and glutamate dehydrogenase, 0.38 mg/ml. All constituents of the assay except TPNH were incubated at 25° for 5 min. At the end of this time TPNH was added. Remaining experimental conditions are given in the legend to Fig. 1.

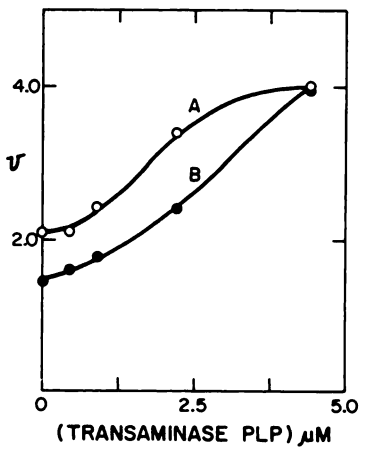

FIG. 4. *Plot of velocity (micromoles per liter per minute) of TPN production with respect to concentration of cytoplasmic glutamate-oxalacetate transaminase-bound pyridoxal phosphate (PLP) in the absence (curve A) and presence (curve B) of 85 μM haloperidol*

Additional experimental conditions were: TPNH, 20 μM; ammonium chloride, 50 mM; pyruvate, 1.0 mM; and glutamate dehydrogenase, 0.5 mg/ml. All constituents of the assay except TPNH were incubated at 25° for 5 min. At the end of this time TPNH was added. Remaining experimental conditions are given in the legend to Fig. 1.

other than pyridoxal phosphate, tightly bound to the transaminase is reductively aminated (13). It has been concluded that this group does not react with glutamate dehydrogenase (13). For example, if pyridoxal phosphate is removed from the transaminase and the apoenzyme is treated with $NaBH_4$, DPNH oxidase activity is lost. However, if the $NaBH_4$-treated apoenzyme is reconstituted with pyridoxamine phosphate, DPNH oxidase activity is not restored, but the reconstituted reduced apoenzyme is twice as reactive with glutamate dehydrogenase as is the original native holoenzyme. Similarly, treatment of the pyridoxamine phosphate form of this enzyme with phenylhydrazine markedly inhibits DPNH oxidase activity but does not alter reactivity with glutamate dehydrogenase. Therefore the group on the transaminase which oxidizes DPNH can apparently be inactivated by these reagents, which react with carbonyl groups without altering or even enhancing reactivity with glutamate dehydrogenase (14). Consequently it is concluded that glutamate dehydrogenase does not react with this group.

As shown in Table 1, high concentrations of perphenazine have no effect on the DPNH oxidase activity of the transaminase.

Reaction with α-ketoglutarate. As shown in Table 1, neither low levels of glutamate dehydrogenase alone nor α-ketoglutarate alone affects the DPNH oxidase activity of the transaminase. However, in the presence of glutamate dehydrogenase, α-ketoglutarate, and transaminase, the rate of DPNH oxidation (6.5 μM/min) is considerably slower than the sum of the rates of the DPNH oxidase reaction alone (4.2 μM/min) plus the glutamate dehydrogenase reaction alone (4.5 μM/min). This suggests that the transaminase inhibits the glutamate dehydrogenase reaction. Inhibition could not be due to

TABLE 1

Interaction among perphenazine, transaminase, glutamate dehydrogenase and low levels of α-ketoglutarate

Assays were performed in the presence of 100 μM DPNH, 50 mM NH_4Cl, 0.025 M sodium arsenate, and 0.1 mM EDTA, pH 7.8, at 25°. The reactions were started with DPNH after a 10-min incubation of the other constituents of the assay at 25°. Initial velocity (V_i) is expressed in units of micromolar concentration of DPN produced per minute. Transaminase concentration is expressed in micromoles per liter of transaminase peptide chain or of transaminase-bound pyridoxal phosphate.

α-Ketoglutarate	Glutamate dehydrogenase	Perphenazine	Glutamate-oxalacetate transminase	V_i
mM	μg/ml	μM	μM	
0	0	0	30	4.2
0	0	100	30	4.2
0.1	0	0	30	4.2
0.1	0	100	30	4.2
0	0.35	0	30	4.2
0	0.35	100	30	4.2
0.1	0.35	0	30	6.5
0.1	0.35	0	0	4.5
0.1	0.35	100	0	3.2
0.1	0.35	100	30	6.0

binding of DPNH to the transaminase, since in the absence of transaminase the concentration of DPNH can be lowered from 100 to 70 μM (a difference equal to the concentration of transaminase peptide chains present) without altering the rate of the glutamate dehydrogenase reaction (26). This inhibition of glutamate dehydrogenase by the transaminase could be due to displacement of α-ketoglutarate from glutamate dehydrogenase by transaminase-bound pyridoxal phosphate. The rate is inhibited because when the level of glutamate dehydrogenase is low transaminase can be bound to this enzyme but cannot be converted to the pyridoxamine phosphate form. Thus the transaminase acts as a competitive inhibitor of α-ketoglutarate. This would explain why, in the presence of perphenazine, glutamate dehydrogenase, transaminase, and low levels of α-ketoglutarate, the rate observed (6.0 μM/min) is essentially equal to the sum of the rates of the DPNH oxidase reaction (4.2 μM/min) plus the glutamate dehydrogenase reaction (3.2 μM/min). That is, in the presence of low concentrations of α-ketoglutarate and both enzymes the rate is essentially the same (6.0 vs. 6.5 μM/min; Table 1) in either the presence or absence of perphenazine. This result again is consistent with the concept that perphenazine has no effect on the enzyme-enzyme complex.

When the level of α-ketoglutarate is sufficiently high (2.0 mM) to displace transaminase from glutamate dehydrogenase, the sum of the rates with either enzyme alone is equal to the rate of the reaction in the presence of both enzymes (Table 2). Under these conditions, therefore, the transaminase is not bound to glutamate dehydrogenase, and perphenazine inhibits the reaction in the presence of both enzymes (velocity is lowered from 10 to 6 μM/min; see Table 2).

Mechanism of perphenazine-transaminase interaction. It has been shown that perphenzine is more inhibitory if it is incubated with glutamate dehydrogenase for at least 4 min and the reaction is then started by adding DPNH than if the drug and enzyme are not incubated. Consequently the above experiments were repeated by first incubating glutamate dehydrogenase with perphenazine and starting the reaction with pyridine nucleotide. Also, more inhibition is produced if a high concentration of glutamate dehydrogenase is incubated with perphenazine, diluted, and assayed than if a smaller amount of enzyme is incubated with the same amount of drug and assayed directly. However, after about 2 min, the velocity of the reaction in the diluted sample begins to increase and approaches that of the undiluted sample (1). These results are consistent with the concept that binding of perphenazine to glutamate dehydrogenase can be described by Eq. 1:

$$E + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EP \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP^* \qquad (1)$$

That is, an enzyme-perphenazine complex (EP) can form rapidly, but this complex slowly forms a more inhibited complex (EP^*) in which the enzyme is more inhibited. Dissociation of EP^* is much less reversible than that of EP. The rate constant k_4 is smaller than k_3, and both are small with respect to k_1 and k_2.

The experiments in this paper demonstrate that perphenazine does not signifi-

TABLE 2

Interaction among perphenazine, transaminase, glutamate dehydrogenase, and high levels of α-ketoglutarate

Assays were performed in the presence of 100 μM DPNH, 50 mM NH_4Cl, 0.025 M sodium arsenate, and 0.1 mM EDTA, pH 7.8, at 25°. The reactions were started with DPNH after a 10-min incubation of the other constituents of the assay at 25°. Initial velocity (V_i) is expressed in units of micromolar concentration of DPN produced per minute. Transaminase concentration is expressed in micromoles per liter of transaminase peptide chain or of transaminase-bound pyridoxal phosphate.

α-Ketoglutarate	Glutamate dehydrogenase	Perphenazine	Glutamate-oxalacetate transminase	V_i
mM	*μg/ml*	*μM*	*μM*	
0	0	0	30	4.2
2.0	0.12	0	0	6.0
2.0	0.12	0	30	10
2.0	0.12	100	0	1.0
2.0	0.12	100	30	6.0

cantly inhibit reactions catalyzed by the enzyme-enzyme complex. This suggests that transaminase might displace perphenazine from glutamate dehydrogenase. To test this possibility, perphenazine was incubated with glutamate dehydrogenase in the presence and absence of transaminase. These solutions were then diluted and assayed for glutamate dehydrogenase activity. If transaminase prevents binding of perphenazine, the *EP* complex should not be formed in the presence of transaminase, and the diluted glutamate dehydrogenase should not be inhibited. If perphenazine is bound in the presence of transaminase, the diluted glutamate dehydrogenase should be inhibited.[3] As shown in Table 3, this is the case. In similar experiments it was found that if 30 μM cytoplasmic transaminase was added to the assay of the diluted, incubated perphenazine–glutamate dehydrogenase solution, the transaminase had no effect on the time course of perphenazine inhibition. This finding is consistent with the results of Table 2, which demonstrated that transaminase has no effect on perphenazine inhibition if the level of α-ketoglutarate is high and transaminase consequently cannot be bound.

TABLE 3

Effect of transaminase on inhibition of glutamate dehydrogenase by perphenazine

Glutamate dehydrogenase (6.3 μg/ml) was incubated alone or with the concentrations of perphenazine and/or cytoplasmic glutamate-oxalacetate transaminase shown below in 0.025 M sodium arsenate and 0.1 mM EDTA, pH 7.8, at 25° for 10 min, then diluted with and assayed in the same buffer. The assay mixture contained 100 μM DPNH, 50 mM NH_4Cl, and 2 mM α-ketoglutarate at 25°. Specific activity is expressed in micromoles of DPN produced per minute per milligram of glutamate dehydrogenase. Transaminase concentration is expressed in micromoles per liter with respect to peptide chains.

Additions to incubation solution	Dilution of incubation solution	Specific activity of glutamate dehydrogenase in assay
None	1:100	47
	1:50	47
Perphenazine (200 μM)	1:100	42
	1:50	29
Glutamate-oxalacetate transaminase (17 μM)[a]	1:100	58
	1:50	61
Perphenazine (200 μM) + glutamate-oxalacetate transaminase (17 μM)	1:100	47
	1:50	22

[a] The slightly faster rate found in the presence than in the absence of transaminase is due to the fact that transaminase protects glutamate dehydrogenase against inactivation.

DISCUSSION

Reactions catalyzed by the complex of glutamate dehydrogenase and transaminase are not significantly inhibited by high concentrations of perphenazine or haloperidol (100–200 μM). These drug concentrations produce maximal inhibition of glutamate dehydrogenase in the presence of high concentrations of α-ketoglutarate (1). It is not practical to use significantly higher concentrations of these two drugs because of their limited solubility in water. Perphenazine and haloperidol were found to be the most potent inhibitors of the reaction with glutamate dehydrogenase and α-ketoglutarate (1). Therefore, since even quite high levels of these drugs do not significantly inhibit reactions catalyzed by the enzyme-enzyme complex and since they are the most potent inhibitors of reactions catalyzed by glutamate dehydrogenase alone, it seems safe to conclude that tranquilizers which markedly inhibit reactions catalyzed by glutamate dehydrogenase alone do not significantly inhibit reactions catalyzed by the glutamate dehydrogenase-transaminase enzyme-enzyme complex.

The ineffectiveness of these drugs in inhibiting the enzyme-enzyme complex is not due to transfer of binding of the drug from glutamate dehydrogenase to the transaminase. In all the experiments reported above, the molar ratio of drug to transaminase peptide chains was quite high, as high as 500:1 in the reaction with TPN and aspartate. Thus binding of drug by trans-

[3] In previous experiments it was shown that DPNH does not alter the amount of inhibition produced by perphenazine when it is incubated with high concentrations of glutamate dehydrogenase and this solution is diluted and assayed (8).

aminase could not deplete the concentration of drug available to inhibit glutamate dehydrogenase. Furthermore, there is no evidence that these drugs are bound to the transaminase. These drugs have no effect on transaminase activity (9) or the DPNH oxidase reaction.

For transaminase to have an effect on inhibition by these drugs, it must be bound to glutamate dehydrogenase. The transaminase apoenzyme (which is not bound; refs. 11, 14) does not alter inhibition by these drugs. Nor does perphenazine affect the transaminase–glutamate dehydrogenase system when the level of α-ketoglutarate is low and an enzyme-enzyme complex can be formed, whereas it is inhibitory when the level of this keto acid is high and an enzyme-enzyme complex cannot be formed. Furthermore, perphenazine has no effect on reactions with oxalacetate and pyruvate when the level of transaminase is sufficiently high to be bound and to displace these keto acids from glutamate dehydrogenase.

Since the transaminase is bound to active sites of glutamate dehydrogenase and perphenazine and haloperidol apparently are not (1, 9), it seems unlikely that the former enzyme simply competes with these drugs for glutamate dehydrogenase. Furthermore, experiments performed with the incubated, diluted perphenazine–glutamate dehydrogenase complex (Table 3) reveal that the transaminase does not prevent binding of perphenazine to glutamate dehydrogenase. These results indicate that the drugs can be bound to glutamate dehydrogenase in the enzyme-enzyme complex but that glutamate dehydrogenase in this complex cannot undergo the drug-induced conformational changes which result in inhibition of the free enzyme (1, 9). Thus transaminase behaves similarly to antibody with glutamate dehydrogenase in preventing conformational changes in glutamate dehydrogenase by low molecular weight ligands (27).

These results could be pharmacologically significant in view of the fact that either the enzyme-enzyme complex or the two separate enzyme-catalyzed reactions (with recycling of α-ketoglutarate and glutamate between the two enzymes) can catalyze tyrosine or aspartate dehydrogenase reactions (14). Which mechanism catalyzes these reactions *in vivo* would depend upon the mitochondrial content of these two enzymes, as it does *in vitro*. These tranquilizers would be expected to inhibit reactions catalyzed by the two separate enzymes (in which α-ketoglutarate and glutamate are transferred between the two enzymes) but not reactions catalyzed by the complex. Thus the levels of these two enzymes in mitochondria from various organs, as well as the concentrations of various coenzymes, purine nucleotides, and substrates, would determine the amount of inhibition produced by tranquilizers. Glutamate-oxalacetate transaminase and glutamate dehydrogenase are located in the same regions of the mitochondrion (28), but the relative ratios of these two enzymes vary in different organs (7–9). Moreover, mitochondrial glutamate-oxalacetate transaminase is the major or only tyrosine aminotransferase in mitochondria (14–16). The enzyme-enzyme complex can be as efficient in catalyzing tyrosine dehydrogenase reactions as the classical recycling of α-ketoglutarate and glutamate between the two enzymes (14). However, the enzyme-enzyme complex is not inhibited by the tranquilizers studied here. This finding could be significant in brain mitochondria.

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