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Inhibitory Actions of the Antidepressant/Antipanic Drug Phenelzine on Brain GABA Transaminase

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Brain GABA transaminase is inactivated by preincubation with antidepressant/antipanic drug phenelzine (β-ethylphenylhydrazine) (mixing molar ratio 10:1) at pH 7.4. The reaction of enzyme with phenelzine was monitored by absorption and fluorescence spectroscopic methods. The inactive enzyme was fully reconstituted by addition of cofactor pyridoxal-5-phosphate. This result implies that the blocking of 1 mol of pyridoxal-5-phosphate per enzyme dimer is needed for inactivation of the enzyme. The time course of the reaction is significantly affected by the substrate α-ketoglutarate, which afforded complete protection against the loss of catalytic activity. The kinetic studies shows that phenelzine reacts with the cofactor of enzyme with a second-order rate constant of 2.1×10^3 $M^{-1}s^{-1}$. It is postulated that the antidepressant/antipanic drug phenelzine is able to elevate the neurotransmitter GABA levels in central nervous system by inhibitory action on GABA degradative enzyme GABA transaminase.

Key words : Brain GABA transaminase, Antidepressant/antipanic drug, Phenelzine

INTRODUCTION

γ-Aminobutyric acid (GABA) is present in many tissues of mammalians and is believed to be a major inhibitory chemical neurotransmitter in the central nervous system, in which it is present with competitive amounts (Fletcher and Fowler, 1980). The release of GABA from nerve terminals and its subsequent binding to its receptor must be followed by a rapid inactivation of the transmitter. The metabolic degradation of GABA is mediated by the action of GABA transaminase which catalyzes the reversible transamination of the neurotransmitter GABA in mammalian brain to yield succinic semialdehyde and glutamic acid. The reaction depends on the presence of pyridoxal-5-phosphate (PLP) at the active site, where it is tightly bound. Succinic semialdehyde is rapidly metabolized by the action of succinic semialdehyde dehydrogenase to succinic acid.

The observation that inactivation of GABA transaminase in brain tissues increase the concentration of neurotransmitter supports the fact that this enzyme exerts a controlling influence on GABA levels (Fletcher and Fowler, 1980). Moreover, the irreversible inhibition of GABA transaminase by a chemical analog of GABA is the basic mechanism of action of drugs used in the treatment of convulsive disorders (Lippert *et al.*, 1977). Further advances in the design of effective drugs for the treatment of epilepsy depend upon detailed information of the target enzyme.

During the last decade, the evidence has accumulated to suggest that GABA may play a role in mood disorders (Lloyd *et al.*, 1989). The most direct evidence for the involvement of GABA in depression results from studies of cerebrospinal fluid (CSF), of which seven of eight reports found low GABA levels of CSF in patients with depression (Berrettini *et al.*, 1984; Bowers *et al.*, 1980; Gerner and Hare, 1981; Gerner *et al.*, 1984; Gold *et al.*, 1980; Kasa *et al.*, 1982; Post *et al.*, 1980; Zimmer *et al.*, 1981). Other abnormalities of GABA function have also been reported, including low activity of both the GABA synthesizing (glutamate decarboxylase) and the catabolizing (GABA transaminase) enzymes in blood of depressives (Berretini *et al.*, 1980, Kaiya *et al.*, 1982).

Even though the antidepressant/antipanic drug phenelzine, known as monoamine oxidase inhibitor, and the tricyclic antidepressant desipramine when administered acutely at high dose have been reported

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to produce an elevation of rat brain GABA levels and GABA transaminase activity of the brain crude homogenates (Popov and Matthies, 1969; Perry and Hansen, 1973), the molecular mechanism of phenelzine effect on GABA levels has not been studied yet. Therefore, we report here inhibitory effect of antidepressant drug phenelzine on purified brain GABA transaminase.

MATERIALS AND METHODS

Materials

Fresh bovine brain was obtained from Majangdong Packing Company in Seoul, Korea. Succinic semialdehyde, NAD^+, PLP, α-ketoglutarate, phenelzine, imipramine, desipramine, and tranylcypromine, were purchased from Sigma (St.Louis, MO, USA). Superose-6, CM-Sepharose, Blue-Sepharose, CM-Sephadex, DEAE-Sephadex and hydroxyapatite were obtained from Pharmacia (Uppsala, Sweden).

Purification of bovine brain GABA transaminase

The purification of bovine brain GABA transaminase was performed by a method developed in our laboratory (Choi *et al.*, 1993). Succinic semialdehyde dehydrogenase was prepared from bovine brain by a combination of CM-Sepharose, Blue-Sepharose and hydroxyapatite chromatographic methods (Lee *et al.* 1995). Protein concentration was determined with a protein assay kit from Bio-Rad using bovine serum albumin as a standard (Bradford, 1976).

Enzyme assay of GABA transaminase

A coupled assay system consisting of two purified enzyme, i.e., GABA transaminase and succinic semialdehyde dehydrogenase were used to study the catalytic conversion of GABA into succinic semialdehyde. Enzymatic assays were performed in 0.1 M sodium pyrophosphate buffer, pH 8.4, containing 5 mM NAD^+, 20 mM GABA, and 10 mM α-ketoglutarate. The progress of the reaction was monitored by measuring the absorbance changes at 340 nm due to the reduction of NAD^+. The coupled assay system is appropriate to measure the rate of transamination when the concentration of succinic semialdehyde dehydrogenase is at least five-fold greater than that of GABA transaminase. One unit of GABA transaminase was defined as the amount of enzyme which produces 1 μmol of succinic semialdehyde per min at 25°C.

Polyacrylamide gel electrophoresis

Discontinuous polyacrylamide gel electrophoresis was performed by the method of Davis (1964). Sodium dodecyl sulfate polyacrylamide gel eletrophoresis was carried out by the method of Laemmli (1970).

Spectroscopy

Spectrophotometric measurements were carried out in Kontron UVIKON Model 930 double beam spectrophotometer. Fluorescence spectra were recorded in a Kontron SFM 25 spectrofluorometer. The reaction of the enzyme with phenelzine was monitored at 415 nm under pseudo-first-order conditions. The observed rate constant K_{obs} was obtained from a plot of log (A_t-A_∞) versus time according to the following equation (1) :

$$A_t-A_\infty=(A_0-A_\infty)_e^{-K_{obs}\cdot t} \quad (1)$$

where A_t, A_0 and A_∞ are the absorbance values at time t, zero, and ∞, respectively.

RESULTS

Reaction of phenelzine with free pyridoxal-5-phosphate (PLP)

The reaction of the free PLP (40 μM) and the antidepressant drug phenelzine was monitored by measuring the change of absorption spectra. Fig. 1 shows that the absoption peak of free PLP (ε_{max}=388 nm) shifted to the shorter wavelength (ε_{max}=325 nm) by interacting with phenelzine, which is a characteristic feature of a pyridoxamine-5-phosphate (PMP) derivative.

Inhibition of GABA transaminase by phenelzine

Incubation of the enzyme (10 μM) with ten-fold molar excess of phenelzine (100 μM) at 25°C for 30 min resulted in complete inhibition of catalytic activity. The reaction was accompanied by a change in the absorption spectrum of the bound cofactor : the absorbance at 415 nm decreases simultaneously with

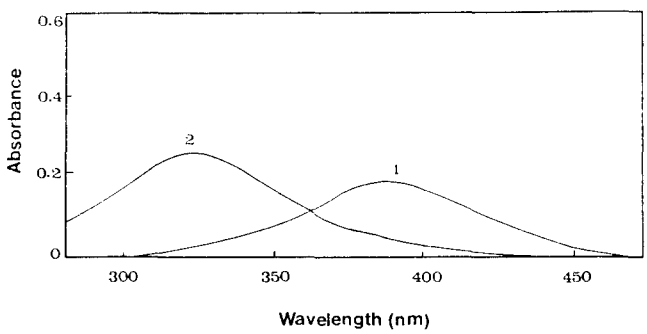

Fig. 1. Absorption spectra of free PLP (40 μM)(1) and the reaction product of PLP with phenelzine (100 μM) after dialysis against 0.1 M phosphate buffer (pH 7.4) (2)

an increase in absorbance at 330 nm (Fig. 2).

The inhibited enzyme was not reactivatied by dialysis against 0.1 M phosphate buffer (pH 7.4). The absorption spectrum of the dialyzed inactive enzyme exhibited an increase in absorption band at 330 nm which might be attributed to the retention of β-ethylphenylhydrazone-pyridoxamine-5-P. The presence of β-ethylphenylhydrazone-pyridoxamine-5-P in the enzyme was measured by fluorescence spectroscopy. The fluorescence emitted by the adduct was significantly quenched upon binding to the enzyme (Fig. 3). But the dissociation of β-ethylphenylhydrazone-pyridoxamine-5-P from the enzyme was achieved by denaturation of the modified enzyme with 6 M guanidium-HCl (Fig. 3).

The catalytic activity of the derivatized enzyme was recovered upon subsequent preincubation of the enzyme with excess of PLP (200 μM) (Table I). To test whether the enzyme saturated with PLP contains more than one molecule of the cofactor involved in catalysis, the reaction of the enzyme sample with phenelzine was allowed to proceed for 10 min, and the absorption spectra were recorded over the wavelength range 330-500 nm. The results of these experiments indicated a decrease in absorbance at 415 nm with a concomitant loss of catalytic activity (Fig. 1 and Table I).

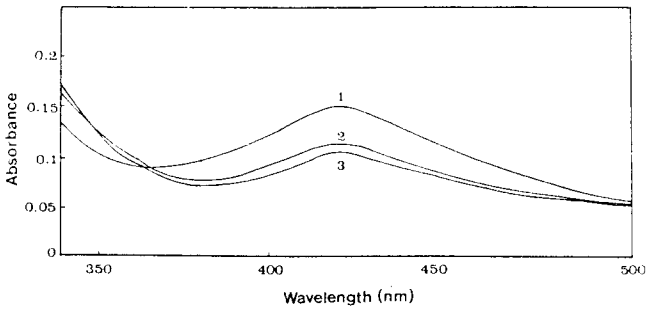

Fig. 2. Absorption spectra of GABA transaminase (1), GABA transaminase (10 μM) reacted with phenelzine (100 μM) (2), and then dialyzed against 0.1 M phosphate buffer (pH 7.4) (3)

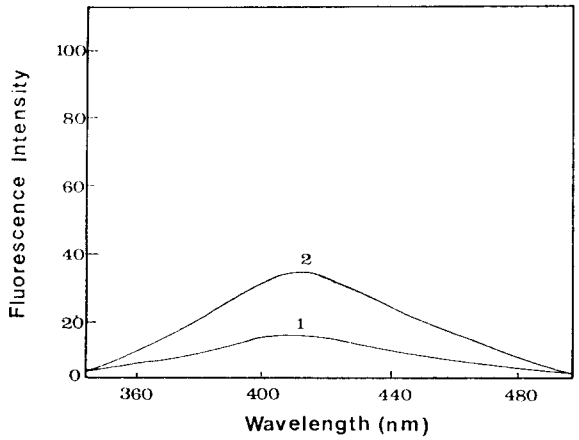

Fig. 3. Emission spectra of GABA transaminase reacted with ten fold molar excess of phenelzine and dialyzed against 0.1 M phosphate beffer (pH 7.4). Spectra were recorded in (1) the absence and (2) presence of 6 M guanidium-HCl (Excitation at 315 nm).

Kinetics of inactivation of GABA transaminase by phenelzine

When the phenelzine (10-100 μM) was incubated with bovine brain GABA transaminase (10 μM) at 25°C, time dependent (1-10 min) inactivation ensued. Inactivation followed pseudo-first-order kinetics with a complete loss of catalytic activity (Fig. 4). A plot of $1/K_{inact}$ versus 1/[phenelzine] yields a value of 33 min^{-1} for the limiting inactivation rate constant. Addition of α-ketoglutarate (5 mM) to incubation mixtures protected the enzyme against phenelzine-induced inactivation, presumably by converting the enzyme to the

Table I. Catalytic properties of native and phenelzine (100 μM) modified GABA transaminase (10 μM)

Enzyme Samples	Mole Adduct per Mole Enzyme	Remaining Activity (%)	K_{cat} (s^{-1})
Native (1 mol pyridoxyl-P/dimer)	0	100	8.4
Native+PLP (2 mol pyridoxyl-P/dimer)	0	100	8.4
Native+Phenelzine→+PLP	0.5	100	8.2
Native+PLP→+Phenelzine	1.0	5	-

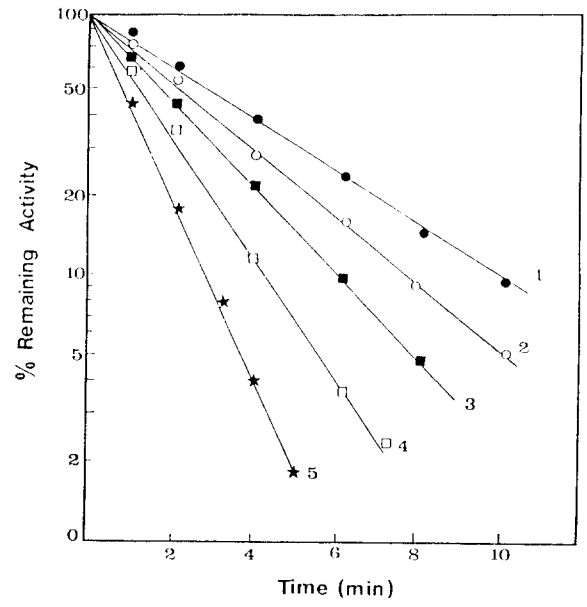

Fig. 4. Inactivation of GABA transaminase (10 μM) by several fixed concentrations of phenelzine : (1), 10 μM; (2), 20 μM; (3), 30 μM; (4), 50 μM; (5), 100 μM

Table II. Inhibition of GABA transaminase activity by phenelzine

Reaction mixture	Specific activity (units/mg)	% specific activity
Enzyme	16	100
Enzyme+phenelzine	0.42	2.6
Enzyme+α-ketoglutarate+phenelzine	14.9	93

Concentrations of enzyme, phenelzine and α-ketoglutarate in reaction mixtures were 0.01, 0.1 and 3 mM, respectively.

nonsusceptible pyridoxamine-5-P form (Table II). The kinetics of the reaction of GABA transaminase with phenelzine was also monitored by spectral changes at 415 nm. When the reaction was measured in the presence of excess of phenelzine, the profile of the reactivity showed a monophasic pattern. A plot of K_{obs} versus phenelzine concentrations yielded a second-order rate constant of 2.1×10^{3} $M^{-1}s^{-1}$. When the enzyme saturated with PLP was allowed to react with 50-fold molar excess of phenelzine, it was observed that pseudo-first order rate constant K_{obs}=0.47 s^{-1} was identical to that determined for the native enzyme. The reactivity curve was monophasic even though more than one molecule of PLP per dimer enzyme reacted with phenelzine.

Effects of tricyclic antidepressant/antipanic drugs on GABA transaminase

Tricyclic antidepressants (Imipramine, desipramine and tranylcypromine) were tested for the possible inhibitory effects on brain GABA transaminase. As shown in Table III, these non-hydrazine tricyclic antidepressant drugs have no effect on brain GABA transaminase.

DISCUSSION

Since abnormal levels of neurotransmitter GABA in brain have been associated with a variety of neurological disorders including epilepsy, seizure, and convulsant disorders, a specific inhibitor of GABA degradative enzyme (GABA transamiase) would be useful in attempts to elevate GABA levels in certain pathological conditions. Recently, it has been reprot-ed that GABA may also play a role in mood disorders (Lloyd *et al.* 1989) and the patients with mood disorders is low plasma GABA levels (Frederic *et al.* 1990). Phenylhydrazine and tricyclic antidepressants (imipramine, desipramine and tranylcypromine), known as monoamine oxidase (MAO) inhibitors, are the most effective pharmacological treatments for panic disorders (Sheehan *et al.* 1980, Sheehan *et al.* 1984; Sheehan *et al.* 1986). Although the mechanism of action responsible for the antipanic efficacy of these agents is unknown, all antidepressant and antianxiety drugs producing antipanic effects also augment GABA transmission (Korf and Venema, 1983; Patel and Schatz, 1975; Lloyd and Pilc, 1984; Lloyd *et al.* 1985; Sheehan *et al.* 1983; Riblet *et al.* 1982). Therefore, in the present work we have examined the mechanism of inhibitory effect of the antipanic/antidepressant drug phenelzine on brain GABA transaminase and postulate the relationship between antipanic, antidepressant and GABA transmission in CNS.

Table III. The effects of tricyclic antidepressant drugs on GABA transaminase

Drug	Concentration (M)	Remaining Activity (%)
Imipramine	2×10^{-3}	100
	5×10^{-4}	95
Desipramine	2×10^{-4}	92
	5×10^{-4}	98
Tranylcypromine	2×10^{-4}	93
	5×10^{-4}	90

The enzyme (10 μM) was preincubated with each drug in 0.1 M potassium phosphate buffer (pH 7.4) at 25°C.

Interaction of the phenelzine with free PLP was shown by the spectroscopic method (Fig. 1). The red shift of the absorption peak suggests that the PLP form reacted with a hydrazine moiety of changes to the PMP form. Phenelzine irreversibly inactivates GABA transaminase by a mechanism-based path resulting in derivatization of the bound cofactor PLP molecule to β-ethylphenylhydrazone-pyridoxamine-5-phosphate. When the native enzyme containing 1 mole of PLP per enzyme dimer reacts with phenelzine, the binding of β-ethylphenylhydrazone-pyridoxamine-5-phosphate (0.5 mole per enzyme dimer) did not influence the ability of the modified inactive enzyme to bind to PLP and regain full catalytic activity, whereas the derivatization of the enzyme saturated with PLP by reaction with phenelzine resulted in the incorporation of more than one mole of β-ethylphenylhydrazone-pyridoxamine-5-P per dimer with concommitant loss of catalytic activity. These results indicate that there exist two reactive sites per enzyme dimer and that both sites bind tightly to the inhibitor β-ethylphenylhydrazone-pyridoxamine-5-P. We have previously reported that the GABA transaminase purified from bovine brain is a dimer with identical subunits (Choi *et al.*, 1993). The enzyme contains 1 mole of tightly bound cofactor PLP per mole of dimeric protein and the homo-dimeric GABA transaminase contains two nonidentical classes of catalytic binding sites and the binding site characterized by a weak affinity for PLP becomes functional after specific chemical modification of the tighly bound PLP (Choi *et al.*, 1993). However, it remains to be es-

tablished whether the two catalytic binding sites differ in their susceptibility to substrate of the enzyme, and to gain more information on the microenvironment surrounding the molecules of cofactor bound to the enzyme.

According to the inhibitory effect of phenelzine on GABA transaminase, we have assumed that the inhibition of this enzyme by phenelzine is important in the elevation of GABA levels in CNS. Phenelzine is frequently employed clinically as an antipanic drug as well as an antidepressant, and it is feasible that its effects on GABA levels may contribute to its efficacy in treating panic disorder (Breshlow *et al.*, 1989). There is now substantial evidence indicating that GABA may play an important role in the actions of antipanic drugs phenelzine but not nonhydrazine type antidepressants. In the cases of imipramine, desipramine and tranylcypromine, all three of which have also been used to treat panic disorder, inhibition of GABA transaminase and elevation of GABA levels are not important component. In summary, the results of the present study supports the hypothesis that the enhancement of the GABA level by inhibitory action of phenelzine on GABA transaminase may be the mechanism explaining antidepressant and antipanic efficacy at this drug.

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