[Chem. Pharm. Bull. 26(3) 853—856 (1978)]

UDC 615.273.3.011.5.015.11.076.7 : 547.466.1.04.09

Effect of Amphetamine on Kyunrenine Aminotransferase and Kynurenine Hydrolase Activities in Normal Mouse Liver

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(Received August 6, 1977)

Amphetamine inhibited (E.C. 2.6.1.7) kynurenine aminotransferase and (E.C. 3.7.1.3) kynurenine hydrolase in mouse liver homogenates. It has been suggested that the inhibitory effect of amphetamine was due to formation of Schiff's base with pyridoxal phosphate, causing a reduction in the level of free pyridoxal phosphate, which is a co-factor for both enzymes. The inhibitory effect of amphetamine for both enzymes is of the competitive type.

Keywords——amphetamine; kynurenine-Aminotransferase; kynurenine hydrolase; pyridoxal-5-phosphate; Schiffs' base formation; competitive inhibition

In a previous report from this laboratory,[2] it was shown that amphetamine forms Schiff's base with pyridoxal-5-phosphate. Bladder cancer caused by aromatic amines became a significant cancer hazard among chemical workers.[3] Patients with bladder cancer have been found to have abnormal tryptophan metabolism.[4–6] The abnormal pattern of urinary metabolites of patients with abnormal metabolism of tryptophan suggested a pyridoxine deficiency.[7] So the present investigation was initiated to study the effects of amphetamine on the kynurenine aminotransferase (2.6.1.7) and kynurenine hydrolase (3.7.1.3) which require pyridoxal-5-phosphate as a coenzyme.

Experimental

Amphetamine sulphate was Smith and Kline Product and was used without further purification. Pyridoxal-5-phosphate (PLP) of high purity over 97% was obtained from Fluka. DL-Kynurenine sulfate, kynurenic acid, anthranilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride and ammonium sulfamate were Koch Light Products. 2-oxoglutarate was purchased from BDH.

Animals used were adult albino mice (Bulb-strain), weighing from 15 to 20 g, fed *ad libitum* on normal animal diet. The animals were sacrificed by exsanguination after being stunned by a blow on the head. The livers were quickly removed and placed in ice-cold 0.1 M potassium phosphate buffer, pH 7.4. The tissues were homogenized in a Potter-Elvehjem homogenizer (1 g/4 ml) based on the wet weight of the tissue. Enzyme assays were performed at 37° according to the method of Amer *et al.*[8] with slight modification. A total volume of 4 ml of 0.1 M phosphate buffer, pH 7.4 containing 30 μmol 2-oxoglutarate, 10.7 μmol DL-kynurenine sulfate, 40 μg pyridoxal phosphate (or as stated), and 0.75 g tissue, equivalent to 3 ml. The reaction was stopped at zero time for the blanks and after 3 hr incubation for test samples by adding 1 ml of 16% trichloroacetic acid to each reaction mixture. The contents were then transferred to centrifuge tubes. Each vessel was washed with 1 ml deionized water, then was transferred to the corresponding centrifuge tube. The mixture was centrifuged at $4000 \times g$ for 10 min at 4°. The supernatants were analyzed.

Kynurenine and anthranilic acid were determined by the Method of Mason and Berg.[9] Kynurenic acid was determined spectrophotometrically by measuring A_{332} followed by substracting the adsorbance of

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kynurenine and anthranilic acid at that wave length. The absorbance was converted to μmol of product formed by referring to standards made according to the Method of Amer *et al.*[8] Enzyme activity was expressed as μmol of product formed per g liver.

Results and Discussion

Table I shows the effect of amphetamine on the production of anthranilic acid (AA) and kynurenic acid (KA) from kynurenine. Increasing the concentration of amphetamine produced a more pronounced inhibition of kynurenine aminotransferase than that for kynurenine hydrolase. Amphetamine at a concentration of 1×10^{-3}M caused about 59% inhibition for kynurenine aminotransferase and only 30% inhibition for kynurenine hydrolase.

Table I. Effect of Increasing the Concentration of Amphetamine Sulphate on the Metabolism of Kynurenine by Normal Mouse Liver Homogenates

Experiment No.	Concentration of amphetamine sulphate (μM)	Concentration of kynurenine (μmol/g liver)[a]	Enzyme activity[b]		% Inhibition KA	% Inhibition AA
			Kynurenine aminotransferase activity	Kynurenine hydrolase activity		
1	0	5.53±0.046	4.88±0.051	0.48±0.024	0	0
2	1×10^{-5}	5.11±0.044	3.8 ±0.04	0.41±0.003	22	14.6
3	1×10^{-4}	4.87±0.033	2.9 ±0.016	0.39±0.01	40	18.8
4	1×10^{-3}	3.95±0.035	2.0 ±0.034	0.34±0.004	59	30

The incubation medium (4 ml) contained 10.7 μM DL-kynurenine sulphate, 30 μM α-ketoglutarate, 160 μg (PLP), and 0.75% whole liver homogenate (3 ml) in 0.1 M potassium phosphate buffer, pH 7.4. Incubations were carried out for 3 hr at 37°.

a) These values represent the difference between the kynurenine recovered and that originally present in the medium.

b) Average values of 4 experiments expressed in μmol/g liver (expressed in μmol of products/g liver).

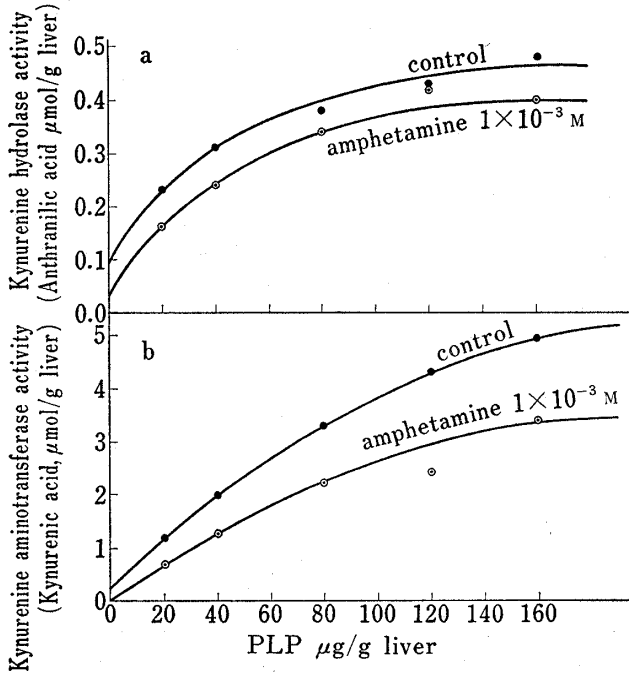

Fig. 1. Changes in Kynurenine Hydrolase and Kynurenine Aminotransferase Activity as a Function of PLP in the Presence and Absence of Amphetamine

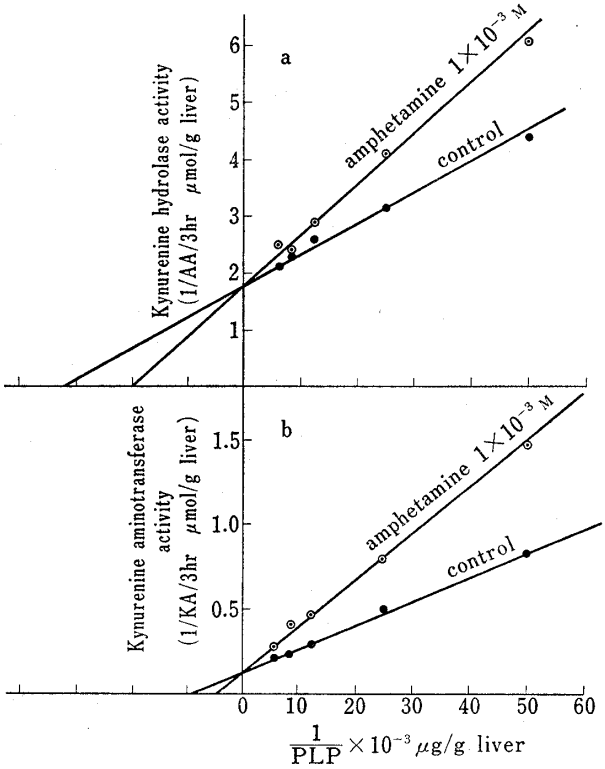

Fig. 2. Lineweaver-Burk Plots for the Effect of Amphetamine on Kynurenine Hydrolase and Kynurenine Aminotransferase

The effect of amphetamine on kynurenine hydrolase and kynurenine aminotransferase in the presence of increasing concentration of pyridoxal phosphate (PLP) is shown in Fig. 1. At a concentration of 1×10^{-3}M amphetamine caused a reduction in the production of AA, and KA. Yet, the inhibition for the kynurenine aminotransferase is more pronounced than that of the kynurenine hydrolase activity.

From Fig. 1 (a and b) the results indicate that in the absence of amphetamine, pyridoxal phosphate stimulated the conversion of kynurenine to AA and KA by kynurenine hydrolase and kynurenine aminotransferase respectively.

Since pyridoxal phosphate interacts with kynurenine and polyvalent cation to form a complex which is an intermediate involved in the action of both kynurenine hydrolase and kynurenine aminotransferase,[9-11] different concentrations of pyridoxal phosphate were used in place of the substrate for the kinetic studies. Anthranilic acid and kynurenic acid formed from kynurenine were taken as a measure of the velocity of both enzymes.

Lineweaver-Burk plots for the effect of amphetamine on kynurenine hydrolase and kynurenine aminotransferase were constructed as shown in Fig. 2 (a,b) respectively. Both plots indicated that amphetamine is a competitive inhibitor to both enzymes.

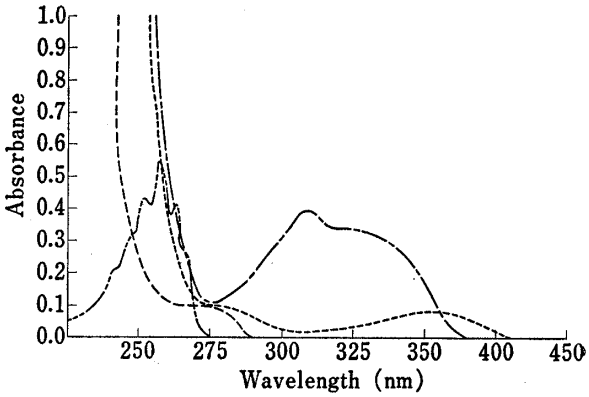

Fig. 3. The Spectra of Anthranilic, Kynurenic Acids, Kynurenine and Amphetamine at pH= 1.06

--------: arthranilic acid (1.0 μmol/6 ml),
————: kynurenic acid (1.0 μmol/6 ml),
.....: kynurenine (1.0 μmol/6 ml),
- - - - - -: amphetamine sulfate 3×10^{-3} M pH=1.06.

Fig. 3 shows the absorption spectra of anthranilic and kynurenic acids and kynurenine at pH 1.06, where these metabolites were checked. The figure illustrates the possible interference between these metabolites. The UV spectrum of aqueous amphetamine solution 3×10^{-3}M at pH 1.06, *i.e.* the pH of measuring the metabolites. Amphetamine shows no absorption at 332 nm. This indicates that Amphetamine does not interfere with absorption of any of the metabolites measured.

Several metabolic intermediates in the metabolism of tryptophan to niacin, are reported to be potential bladder carcinogenic.[12-14] It was also reported that observed lack of pyridoxal phosphate in the infested liver with *Schistosoma manosoni* resulted in the modified levels of kynurenine matabolites,[15] and induced functional B_6 deficiency in man host.[16] It was also reported that antimony tartarate caused a change in the relative concentration of the different metabolites in the tryptophan metabolism.[17] It was suggested that this might be related to the development of bladder tumors in the bilharzial patients treated with the drug. This was attributed to functional pyridoxal phosphate deficiency.

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It is suggested that amphetamine-pyridoxal complex may compete with pyridoxal-substrate complex for the active site of both enzymes. It could also be that amphetamine caused a deficiency of the pyridoxal in the tissue by forming a Schiff base,[2] so the amount of pyridoxal phosphate available is reduced. This will affect the level of PLP-substrate complex.

From those investigations reported here, it seems that pyridoxal phosphate is a factor directly responsible for the observed inhibition to the two enzymes, and that amphetamine is not only a stimulant drug and acts on the peripheral sympathetic nervous system, but also could affect the tryptophan metabolism in the body, causing accumulation of undesirable metabolites formed through kynurenine pathway which may be bladder carcinogens.