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EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON THE DISTRIBUTION OF TRYPTOPHAN IN APO-TRYPTOPHAN PYRROLASE LACKING SPECIES

Darakhshan, J.H. M.A. Haleem, and S.N. Hasnain

Department of Biochemistry, University of Karachi, Karachi-32, Pakistan

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1. Chronic ethanol administration inhibited tryptophan pyrrolase activity in an apoenzyme lacking species like rabbit by causing decrease in substrate/product ratio. 2. Tryptophan enhancement of enezme activity in control and ethanol treated rabbits again showed in inhibition in the latter. 3. The conconcentration of tryptophan in plasma and of tryptophan and 5-hydroxy tryptamine in brain were significantly decrease in ethanol treated rabbits.

INTRODUCTION

Studies on tryptophan metabolism in mammals have indicated that the oxidation of tryptophan via kynurenine pathway, located in liver is quantitatively the most important pathway of tryptophan metabolism [1,2]. The tryptophan pyrrolase located in liver controls the metabolism of tryptophan via this pathway [3,4]. Studies on the effect of chronic ethanol administration on tryptophan pyrrolase activity in rats have been reported to decrease the activity of haem free predominant form or apoenzyme thereby altering the availability of circulating tryptophan to the brain [5,6]. Tryptophan pyrrolase activity has been measured in the liver of various animal species, and absence of apoenzyme has been reported in few species, including rabbit [5].

We measured the effect of ethanol administration on tryptophan pyrrolase activity in an apoenzyme lacking species, the the rabbit, to observe changes in brain tryptophan metabolism.

MATERIALS AND METHODS

Chemicals. 5-Hydroxy tryptamine creatinine sulphate complex, 5-hydroxy indol-3-yl acetic acid and l-kynurenine sulphate were purchased from Sigma Chemical Company USA. *l*-tryptophan, *o*-phthaladehyde and other chemicals were either from BDH Chemicals Pool England or from E. Merck Dermstadt. *Animals and treatment:* Locally bred male rabbits (1300-1700 g) were used for the experiment. Ethanol was added in drinking water of test animals in concentration of 5% for initial two days, 7.5% for two more days and 10% for final eleven days. An intraperitoneal injection of tryptophan (500mg/kg) was given to some ethanol treated or normal rabbits two hours before sacrifice. An equal volume of 0.9% (w/v) NaCl was injected intraperitioneally to the respective controls.

Control and test rabbits were caged separately. It was found that the volume of fluid (water by control animals and ethanol by test animals) drink by control group and test group did not differ significantly.

Determination of Tryptophan Pyrrolase Activity. The activity of tryptophan pyrrolase was determined in liver homegenates by measuring the formation of kynurenine from *l*-tryptophan [3]. Liver was removed within 10s of the sacrifice of animal and was homegenised for 1 min at 1100 rev/ min in 7 volume of solution (140mM KCI in 2.5mM NaOH) at 0°C. Substrate solution was prepared by mixing 30 ml of 0.005M *l*-tryptophan and 0.2M phosphate buffer pH, 7. Substrate solution was incubated at 37°C prior to the addition of homogenate. The homogenate (15ml) as soon as prepared was added to the substrate solution and reaction mixture incubated at 37°C with shaking (180 oscillations/min). Samples (3ml) of the reaction mixture were taken out an interval of 15min. The reaction at appropriate time interval was stopped by addiong 2ml of freshly prepared metaphosphoric acid (15% w/v). The content was shaken for another 3 min and filtered. 1.5ml of NaOH (0.6 M) was added to 2.5 ml of filtrate and kynurenine present was determined by measuring E_{365} with Bausch and Lomb spectronic 21. Concentration (μmol) of kynurenine was calculated by taking ξ = 4540 litre mol^{-1} cm^{-1}. The μmol of kynurenine produced /gm of liver were plotted against time and the enzyme activity (μmol of kynurenine produced h/g of liver) was determined from the slope.

Estimation of 5-hydroxy Tryptamine, 5-hydroxy Indol-3-yl Acetic Acid and Tryptophan. 5-hydroxy tryptamine

and 5-hydroxy indol-3-yl acetic acid were estimated in whole brain by spectrofluorimetric method of Curzon and Green [7]. Tryptophan was estimated in plasma, plasma dialysate, liver and brain homogenates by spectrofluorimetric method of Dewey and Denckla [8] revised by Bloxam and Warren [9] using Schimadzu fluorescene monitor RF 500 LC.

RESULTS

The effect of chronic ethanol administration on tryptophan level in plasma, liver and brain is shown in Table 1. Chronic ethanol administration for two weeks has been found to decrease total tryptophan concentration by 34.3% in plasma and 15.78% in brain (Table 1). Tryptophan concentration in liver (8.69% decrease) was not significantly altered ($P>0.1$).

The measurement of tryptophan pyrrolase activity in liver of rabbits showed that the enzyme activity remained unaltered (0.536 ± 0.05) in presence or absence of added haematin. The tryptophan pyrrolase activity in chronically ethanol treated rabbits was 30.67% less than the normal rabbits ($P<0.05$) (Fig. 1a and 1b). Intraperitoneal injection of tryptophan (500mg/kg) enhanced enzyme activity both in control and ethanol treated rabbits by 267.2 and 278.9% respectively ($P<0.0005$). The enzyme acitivity (1.408 ± 0.06) in liver of ethanol treated rabbits receiving an intraperitoneal injection of tryptophan was still less than the enzyme activity (1.968 ± 0.109) in liver of control rabbits receiving an intraperitoneal injection of tryptophan (Fig. 2a and 2b, Table 2). The basal kynurenine level in ethanol treated rabbits was greater than the basal kynurenine level in control rabbits (Fig. 1a and 1b).

Chronic ehtanol administration was found to decrease 5-hydroxy tryptamine concentration in brain by 19.35% ($P<0.05$), (Table 1). The decrease in 5-hydroxy indol-3-yl acetic acid concentration in ethanol treated rabbits was not very significant ($P>0.1$).

DISCUSSION

Studies on the effect of chronic ethanol administration on tryptophan metabolism in rats [6] have shown an in-

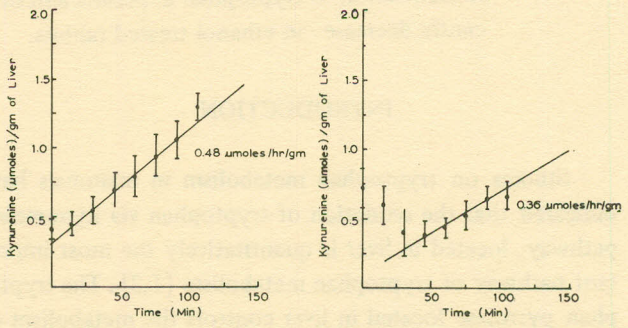

Fig. 1. Effect of chronic ethanol administration on tryptophan pyrrolase activity in rabbits. a. Control animals. b. Ethanol treated animals. Ethanol was administered orally for two weeks. The enzyme activity was determined as descreibed in Methods and Materials section. Each point represents mean value for 6 rabbits ± S.E.M.

Table 1. Effect of chronic ethanol administration on tryptophan pyrrolase activity, concentration of tryptophan in plasma, liver and brain, concentration of 5-hydroxy tryptamine and 5-hydroxy indol-3-yl- acetic acid in brain of rabbits. Results are expressed as mean ± S.E. of six animals. Ethanol was administered orally.

Determination	Control animals	Ethanol treated animals	% decrease	P value
Tryptophan pyrrolase activity (µmol of kynurenine formed /hr/g liver	0.536 ± 0.059	0.371 ± 0.049	30.67%	<0.05
Plasma tryptophan (µg/ml)				
Total (µg/ml)	16.288 ± 2.96	10.70 ± 1.82	34.3%	<0.05
Free (µg/ml)	3.275 ± 0.55	1.570 ± 0.27	52.0%	
Free %	20.605 ± 2.11	14.705 ± 1.14	28.6%	<0.05
Liver tryptophan (µg/g)	5.425 ± 0.10	4.950 ± 0.46	8.67%	>0.1
Brain tryptophan (µg/g)	2.470 ± 0.36	2.080 ± 0.27	15.7%	<0.1
Brain 5-hydroxy tryptamine (µg/g)	0.587 ± 0.03	0.473 ± 0.04	19.35%	<0.05
Brain 5-hydroxy indol-3-yl acetic acid (µg/g)	0.387 ± 0.05	0.368 ± 0.05	5.07%	>0.1

crease in brain tryptophan metabolism associated with a decrease in tryptophan pyrrolase (apoenzyme) activity.

The present paper is concerned with the effect of chronic ethanol administration on liver tryptophan pyrrolase activity and hence on brain tryptophan metabolism in an apoenzyme lacking species the rabbit. Liver tryptophan pyrrolase activity in absence of added haematin (holoenzyme) is shown in Fig. 1a, Table 1. The enzyme acitivity

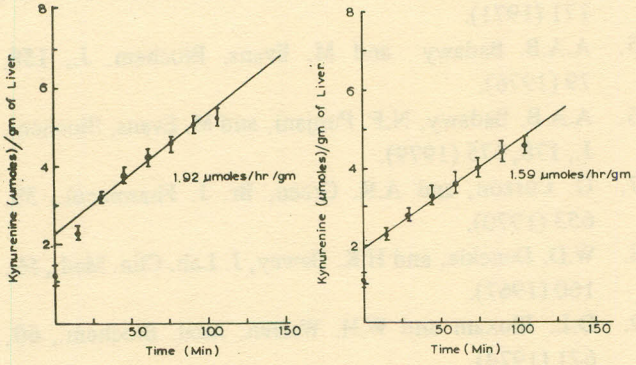

Fig. 2. Effect of tryptophan loading on chronic ethanol induced changes of tryptophan pyrrolase activity in rabbits. a. Control animals. b. Ethanol treated animals. Ethanol was administered orally for two weeks. Tryptophan (500mg/kg) was injected intraperitoneally two hr. before sacrifice. The enzyme activity was determined as described in Methods and Materials section. Each point represents mean value ± S.E.M.

in presence of added haematin i.e. total enzyme acitivity remains unaltered (results not shown) showing absence of apoenzyme acitivity in rabbits. In Table 1, Fig. 1 the holoenzyme activity which is the total enzyme activity in rabbits is shown as μ mol of kynurenine formed/h/g wet of liver. The intraperitoneal administration of tryptophan enhanced holoenzyme activity by 267.2% (Table 2, Fig. 2a).

The tryptophan enhancement of pyrrolase activity has been studied in various animal species. A large number of species except catfish [10] and steer [11] have been found to show an enhancement of the enzyme activity. We selected an apoenzyme lacking species for studies because, it is apoenzyme which is primarily inhibited by chronic ehtanol administration [6]. Chronic ethanol administration in apoenzyme lacking species may show direct effect on tryptophan metabolism.

Tryptophan pyrrolase activity in control and ethanol treated rabbits are shown in Table 1 and Fig. 1a, b. The results show an inhibition of enzyme activity in ethanol treated rabbits by 30.67%. Tryptophan enhancement of enzyme activity is observed both in control and ehtanol treated rabbits (Fig. 2a, 2b). We thus report an inhibition of holoenzyme activity in liver of ethanol treated treated rabbits. The inhibition of tryptophan pyrrolase activity by chronic ethanol treatment in paoenzyme possesing species, the rat [6], may however be greater.

A comparision of Fig. 1a and 1b shows a high kynurenine concentration in ethanol treated rabbits. The inhibition of holoenzyme in ethanol treated rabbits is probably due to accumulation of a large amount of kynurenine. A low substrate/product ratio may be responsible for decreased tryptophan pyrrolase activity in ethanol treated rabbits. The tryptophan enhancement of enzyme activity in ethanol treated rabbits and control rabbits may again be explained by an increased substrate/product ratio. Unlike rates [6] chronic ethanol administration for two weeks was found to decrease total tryptophan concentration in plasma, liver and brain of rabbits (Table 1). It is established that quantitatively the most important route of tryptophan metabolism is via kynurenine pathway, located in liver [2]. This quanititiatively important pathway is inhibited in ethanol treated

Table 2. Tryptophan enhancement of tryptophan pyrrolase activity in liver of normal and chronic ethanol treated rabbits. Tryptophan (500mg/kg/ml) was injected intraperitoneally for tryptophan enhancement. Control rabbits received an equal volume of 0.9% (w/v) NaCl. Ethanol was administered orally in drinking water. Results are expressed as mean ± S.E. of six animals.

Determination	Control animals		Ethanol treated animals	
	NaCl injected	Tryptophan injected	NaCl injected	Tryptophan injected
Tryptophan pyrrolase activity (μmol of kynurenine formed /h/g	0.536 ± 0.059	1.968 ± 0.109	0.371 ± 0.049	1.408 ± 0.066
% increase		267.2%		278.9%
P value		<0.0005		<0.0005

rabbits (Table 1). The decrease of total tryptophan concentration in plasma and liver, with a simultaneous decrease of tryptophan pyrrolase activity in ethanol treated rabbits (Table 1) shows the enhancement of tryptophan metabolism by a route other than kynurenine pathway. Another pathway of tryptophan metabolism is via its conversion to 5-hydroxy tryptamine. Brain tryptophan hydroxylase, the rate limiting enzyme of 5-hydroxy tryptamine synthesis exists unsaturated with its substate, the tryptophan [12,13]. Brain tryptophan concentration therfore plays as important role in the synthesis of 5-hydroxy tryptamine. Brain tryptophan concentration however depends upon the plasma tryptophan concentration. As is evident from Table 1 in chronic ethanol treated rabbits the decrease in total plasma tryptophan concentration is 34.2% while decrease in brain tryptophan concentration is only 15.7%. The results thus indicate that chronic ethanol administration in an apoenzyme lacking species like the rabbit inhibits tryptophan pyrrolase (holoenzyme) activity. The decrease of tryptophan pyrrolase activity should result in an increase of total tryptophan concentration in plasma, liver and brain. The decrease of total tryptophan concentration in plasma, liver and brain associated with decreased hepatic catabolism of tryptophan suggests an increase in tryptophan utilization in gluconeogenesis or in protein synthesis. Futher work involving the measurement of above two in ethanol treated rabbits should be interesting.

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