Biological Sciences Section

Pakistan J. Sci. Ind. Res, Vol. 31, No. 5, May 1988

EFFECT OF ETHANOL ON BRAIN AND HEPATIC TRYPTOPHAN METABOLISM IN RABBIT

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(Received September 29, 1987; revised June 18, 1988)

Acute ethanol administration increases serum free tryptophan but decreases total tryptophan concentration Tryptophan pyrrolase activity is decreased after two hours, brain tryptophan and 5 hydroxy tryptamine concentrations are increased. It is suggested that ethanol enhances brain tryptophan metabolism because it alters the availability of circulating tryptophan to the brain and decreases its hepatic catabolism.

Key words: Ethanol, Brain, Tryptophan.

INTRODUCTION

Acute ethanol administration enhanced the liver tryptophan pyrrolase activity in rats by a co-factor type mechanism [1] and by increasing the availability of circulating tryptophan to the brain [2,3]. Chronic ethanol administration inhibits the tryptophan pyrrolase activity in rabbits due to a low substrate product ratio [4]. Decreased hepatic catabolism with increased serum free and brain tryptophan concentrations, increases the L-tryptophan metabolism in brain [5]. Present work describes the effect of ethanol on brain tryptophan metabolism in the absence of increased hepatic catabolism. Rabbit is an apo-enzyme-lacking species [6] therefore it is selected for the study.

MATERIALS AND METHODS

Rabbits about 3 to 5 month of age having weight 0.9 - 1.4 kg irrespective of sex were kept in the animal room. 25% ethanol was given intraperitoneally (5 ml/kg). 25% ethanol was prepared in 0.9% NaCl. Control animals received an equal volume of 0.9% NaCl. Animals were sacrificed two hours after the injection and samples of serum, liver and brain were collected. Tryptophan pyrrolase activity was determined by the method of Feigelson and Greengard [7] as described by Darakhshan *et al* [4]. Concentrations of total tryptophan in serum, liver and brain homogenate and concentration of free tryptophan in serum dialysate were determined by the spectrofluorimetric method of Denkla and Dewey [8] revised by Bloxam and Warren [9]. Concentration of 5-hydroxy tryptamine was estimated in whole brain by spectrofluorimetric method of Curzon and Green [10]. The concentration of amino acid nitrogen in serum dialysate was determined by nin-hydrin method of Khachadurian *et al* [11] and Siest and Besson [12].

RESULTS

The results on the effect of intraperitoneal administration of 25% ethanol (5ml/kg) on tryptophan level in serum, liver and brain, liver tryptophan pyrrolase activity, concentration of 5HT in brain and amino acid nitrogen in serum dialysate are given in Table 1. Ethanol administration increased the serum free tryptophan, brain tryptophan and brain 5HT by 31.8% ($P < 0.0005$), 60.8% ($P < 0.05$) and 16.1% ($P < 0.01$) respectively and decreased concentration of total tryptophan in serum and liver by 47.4% ($P < 0.0005$) and 18.5% ($P < 0.005$). Acute ethanol administration was found to inhibit tryptophan pyrrolase activity by 33.3% ($P < 0.05$). The concentration of amino acid nitrogen was decreased by 40.6% ($P < 0.05$) (Table 1).

DISCUSSION

The increase in the serum free tryptophan concentration (Table 1) indicates that this is due to the sequence of reactions involving the catechol amino mediated lipolysis which is followed by the displacement of serum bound tryptophan [6]. Acute ethanol administration enhances the enzyme activity [1,2,3] in rats. However acute ethanol administration decreases tryptophan pyrrolase activity in rabbits (Table 1). The contradictory effect of acute ethanol administration in rats and rabbits liver tryptophan pyrrolase activity may be due to the difference in tryptophan pyrrolase in these species. Rat is an apo-enzyme possessing where as rabbit is an apo-enzyme lacking species.

The results (Table 1) indicate that an inverse relationship exists between serum and total tryptophan. This gives strong evidence to support that the free tryptophan is available for the uptake by brain and other tissues and thus for intracellular metabolism is general [3]. Therefore when the free tryptophan increases these processes may also be

enahnced so that equillbrium results in the release of tryptophan from the bound form and decrease in total tryptophan concentration. The increase in serum free tryptophan concentration (Table 1) causes a simultaneous increase in brain tryptophan (Table 1) as a result of which total tryptophan concentration show 47.4% decrease (Table 1). Change in the brain tryptophan concentration play an important role in the synthesis of 5-hydroxy tryptamine. Tryptophan hydroxylase is unsaturated with its substrate [14,15]. Therefore increase in brain tryptophan concentration (Table 1) increases the synthesis of 5HT (Table 1).

The effect of ethanol on brain 5HT synthesis has been reported to be due to the change in availability of circulating tryptophan [5]. The increase in availability of tryptophan to the brain is due to the decreased liver tryptophan pyrrolase activity [5,16]. Our results strongly support the previous finding that decreased tryptophan pyrrolase activity (Table 1) enhances the brain 5HT synthesis (Table 1) on acute ethanol administration in rabbits. Inhibition of rat brain metabolism by ethanol withdrawal [17] further explains the involvement of tryptophan pyrrolase activity in brain tryptophan metabolism.

It has been found [18,19,20] that brain tryptophan is not the reflection of serum free tryptophan only, but the ratio of this amino acid to plasma large neutral amino acid that compete with the tryptophan for the up-take by brain. The present work indicates that ethanol decreases the concentration of amino acid nitrogen (40.6%) whereas it causes an increase in serum free tryptophan concentration. If we measure only the tryptophan nitrogen then it is likely that the nitrogen content will be increased in

Table 1.

Determination	Control	Ethanol treated	% change	P value
Serum total tryptophan (μ gm/ml)	7.48 ± 0.46	3.93 ± 0.43	47.4% decrease	< 0.0005
Serum free tryptophan (μ gm/ml)	0.44 ± 0.06	0.58 ± 0.08	31.8% increase	< 0.0005
Liver tryptophan (μ gm/gm)	8.13 ± 0.23	6.62 ± 0.47	18.5% decrease	< 0.005
Brain tryptophan (μ gm/gm)	2.25 ± 0.20	3.62 ± 0.35	60.8% increase	< 0.05
Tryptophan pyrrolase activitity (μ mol of kynurenine formed/ hr/gm liver)	1.8 ± 0.35	1.2 ± 0.25	33.3% decrease	< 0.05
5 hydroxy tryptamine (Brain μ gm/gm)	0.62 ± 0.06	0.72 ± 0.08	16.1% increase	< 0.01
Amino acid nitrogen (mg/100 ml)	1.91 ± 0.30	1.13 ± 0.17	40.6% decrease	< 0.50

Effect of ethanol on concentration of tryptophan in serum, liver and brain, concentration of 5 hydroxy-tryptamine in brain, tryptophan pyrrolase activity in liver and concentration of amino acid nitrogen in serum dialysate. Results are express as mean ± S.E. of 10 animals (except in case of Tryptophan pyrrolase activity which is expressed as mean ± S.E. of 4 animals.

dialysate. This decrease in amino acid nitrogen content indicates that ethanol decreases the concentration of that amino acid which competes with the tryptophan for the uptake by brain.

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