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Effect of Drug on Tryptophan-Protein Binding in Plasma, Liver and Brain of Rabbit

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Present study has been made to investigate the chronic ethanol treatment of rabbits, produced a decrease in total tryptophan concentration by 45.25 % in plasma, 32.58 % in brain and 3 % in liver respectively. The decrease in total tryptophan utilization in plasma may related to enhanced tryptophan utilization by brain or liver. The effect of tryptophan load on plasma, brain and liver was compared in controls and ethanol treated rabbits. The control and ethanol treated rabbits received an injection of tryptophan (500 mg/100 g body weight, imp.) 2 h before experimentation. Tryptophan concentration increased by 302.35 % in plasma, 809.64 % in brain and 552.80 % in liver. In the ethanol treated rabbits tryptophan loading caused an increase of total tryptophan concentration by 2121.65 % in plasma, 2191.63 % in brain and 695.55 % in liver. The results suggested the role and possible mechanism involvement in the metabolism of serotonin following ethanol treatment.

Key Words: Rabbit, Tryptophan, Ethanol, Serotoinin.

INTRODUCTION

Tryptophan is one of the essential amino acids which is obtained by the body from ingested protein[1-7]. Normally the ingested tryptophan is almost totally absorbed from gastrointestinal tract, even in doses as high as 100 mg/kg[8,9]. After absorption its fate depends on many factors. There is some evidence that tryptophan may play a special role in the regulation of protein synthesis in the liver and possibly at other sites in the body[10-16]. It is likely that because of activity of the metabolites of tryptophan the body has envolved a mechanism, which finally regulates the concentration of

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free tryptophan. Tryptophan in plasma is mostly (*ca.* 90 %) bound by albumin[17]. It is bounded to a slight degree to some other plasma proteins[18]. The role played by the various physiological factors in the regulation of the ratio of free to bound tryptophan is not clearly understood. Free fatty acids regulates free tryptophan concentration in the plasma by displacing it from binding sites[19-21]. Similarly drugs displaces tryptophan from its binding sites[22]. Many other drugs (proben acid and clofibrate) are bound by albumin clofibrate, while lowering both total and bound tryptophan increase the amount of free tryptophan[23] proben acid increase free tryptophan in both plasma and brain.

Different Physiological factors such as age and sex as well as various hormones may influence the metabolism of tryptophan by inducing the rate limiting enzyme tryptophan-2,3-monoxygenase (tryptophan pyrrolase)[24-26]. About 1 % of ingested tryptophan normally enters 5-OH tryptamine synthesis pathway, but because of the great biological activity of this compound it is of importance especially in the central nervous system (CNS). Only free unbound tryptophan is able to penetrate the blood brain barrier, this free tryptophan is an important regulator of 5-HT synthesis in central nervous system[27].

Tryptophan is converted to kynurenine in the liver and to serotonin in the brain[28]. Chronic ethanol treatment results in an increase of total tryptophan pyrrolase activity[29]. Chronic ethanol administration of rats has been previously shown to enhance brain 5-HT synthesis by increasing the availability of circulating tryptophan to the brain secondary to NAD (P) H mediated inhibition of liver tryptophan pyrrolase activity[30]. Changes in tryptophan pyrrolase (EC.1.13.11.11) activity and tryptophan load effecting the breakdown of tryptophan by tryptophan pyrrolase have also been studied[31].

6 g load of tryptophan increased brain tryptamine metabolism more than 3 g load also some of the transmitter amine in brain is derived from peripheral sources and diffuse from blood to brain[32]. Tryptophan loading has been shown to increase brain but not plasma tryptophan levels more in ethanol treated rats[33-42].

The objective of present investigation is to show: (i) Effect of chronic ethanol administration on the distribution of tryptophan in plasma, brain and liver of rabbits. (ii) Effect of tryptophan loading on chronic ethanol induced changes of tryptophan metabolism. (iii) To compare tryptophan up takes by brain in chronic ethanol treated rabbits and in chronic ethanol treated rabbits receiving tryptophan injection.

The concentrations of total protein in liver and glucose in plasma of rabbits have been estimated by Biuret and Nelson Somogi methods, respectively.

EXPERIMENTAL

All reagents used were Merck, BDH and AR grades. Male adult rabbits weighing (1-2 kg) were kept on diet and water for 15 d. Ethanol was given freely in drinking water in the concentration of 5 % for 5 d 7.5 % for 5 d more and finally 10 % for remainder of periods. Some control and ethanol treated animals also received an injection of tryptophan (500 mg/100 g body weight) 2 h before being sacrificed.

Tryptophan for injection: Tryptophan (500 mg/100 g body weight) was dissolved in 4 M NaOH. pH adjusted to 7.3 by HCI then volume made upto 20 mL by 0.9 % NaCl, 2 mL/100 g body weight was injected 2 h before being sacrificed.

Determination of tryptophan: The levels of tryptophan were determined by the fluorimetric method[34].

RESULTS AND DISCUSSION

Table-1 shows that the chronic ethanol administration to rabbits produced a decrease in brain tryptophan concentration by 45.25 %. The decrease in liver by 3 % was not significant, while the decrease in total tryptophan concentration in plasma was 32.58 %. The decrease of total tryptophan concentration in plasma suggested enhanced tryptophan metabolism *via* possibly serotonin pathway. The administration of ethanol for 5 d caused no significant changes in the concentration of tyrosine in serum but significantly reduced serum total tryptophan[35].

TABLE-1
EFFECT OF TRYPTOPHAN LOADING ON CHRONIC ETHANOL INDUCED CHANGE OF TRYPTOPHAN CONCENTRATION IN PLASMA, BRAIN AND LIVER OF RABBITS

Experiment	Total plasma (μg/mL)	Free (μg/mL)	Brain (μg/mL)	Liver (μg/mL)
Control	16.53	9.79	7.98	15.32
Ethanol treated	09.05*	4.04*	5.38*	14.86
Tryptophan injected	66.51*	43.24*	72.59*	100.01*
Tryptophan injected and ethanol treated rabbits	201.06*	118.56*	123.29*	118.22*

*P < 0.05 by t-test

Alcoholics had decreased plasma levels of tryptophan, the serotonin precursor and a decrease ratio of tryptophan over amino acids competing for transport into the brain central serotonin deficiency may contribute to the depressive states frequently seen in alcoholics[36].

Long-term treatment with ethanol increased 5-HT metabolism in rats and mice by increasing the activity of the regulatory enzyme tryptophan pyrrolase[37] and/or availability of circulating tryptophan to the brain secondarily to an inhibition of hepatic tryptophan pyrrolase[38]. Conversely

ethanol withdrawal after long-term treatment decreased 5-HT metabolism and tryptophan availability[39]. The results were of interest as evidence in support of a role of 5-HT in depression[40]. It is also clinically recognized that depression and other aspects of lowered mood are associated with alcohol withdrawal.

Administration of tryptophan (500 mg/100g body weight) to control rabbits increases total tryptophan concentration by 302.35 % in plasma and 552.80 % in liver. In the ethanol treated rabbits tryptophan loading involved an increase in total tryptophan concentration by 2121.65 % in plasma and 659.55 % in liver (Table-1). The results show a greater increase of tryptophan in plasma and liver of ethanol treated animals receiving tryptophan injection. The increase in brain tryptophan concentration in control animals receiving tryptophan injection was 809.64 % while it was 2191.63 % in ethanol treated animal receiving tryptophan injection. The findings show enhance brain tryptophan metabolism in chronic ethanol treated rabbits to be due to increased plasma tryptophan concentration, unlike previous study on rats[33].

It is reported here that administration of tryptophan load increases both plasma and brain tryptophan levels more in ethanol treated rabbits. Inconjunction with our previous report[41] the present study implies that tryptophan containing diet may potentiate brain serotonin responses to ethanol.

The results for total tryptophan concentration in plasma, brain and liver of control animals are given in Table-2. The results indicate total and free tryptophan concentration in plasma and total tryptophan concentration in brain and liver.

The total and free tryptophan concentration is found to be 16.53 ± 1.99 and 9.79 ± 1.63 μg/mL in plasma, respectively as shown in Table-2.

Further more the total tryptophan concentration in brain and liver is found to be 7.98 ± 0.95 and 15.32 ± 1.25 μg/g. Free tryptophan concentration is calculated in plasma because it determines tryptophan metabolism at various sites.

Similarly, the results for total tryptophan concentration in plasma, brain and liver of ethanol treated animals according to (Table-3). The results indicate total and free tryptophan concentration is found to be 9.05 ± 0.76 and 4.04 ± 0.51 μg/mL in plasma, respectively. Further more the total tryptophan concentration in brain and liver is found to be 5.38 ± 0.17 and 14.86 ± 1.25 μg/g, respectively.

The results show that chronic ethanol treatment of rabbit produces a decrease in total tryptophan concentration by 45.25 % ($P = 0.05$-0.025) in plasma 32.58 % ($P = 0.05$-0.025) in brain and 3 % ($P > 0.1$) in liver, respectively (Tables 2 and 3). The decrease in total tryptophan turnover in brain or liver.

TABLE-2
EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON TRYPTOPHAN CONCENTRATION IN PLASMA BRAIN AND LIVER OF RABBITS

Control S. No.	Plasma (μg/mL)	Free (μg/mL)	Free (%)	Brain (μg/g)	Liver (μg/g)	Total liver protein (g/g)	Plasma glucose (g/mL)
1	14.14	4.01	28.35	5.98	10.13	0.311	391.24
2	12.24	7.34	59.96	10.24	18.58	0.311	272.99
3	18.43	14.20	77.64	6.80	16.01	0.210	310.48
4	13.44	11.52	85.71	6.04	16.32	0.247	374.82
5	24.40	11.90	48.77	10.88	15.58	0.269	270.00
Mean	16.53	9.79	60.08	7.98	15.32	0.26	323.90
SD	± 4.45	± 3.64	± 20.49	± 2.12	2.79	0.03	50.60
SE	± 1.99	± 1.63	± 9.19	± 0.95	1.25	0.01	22.69

TABLE-3
EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON TRYPTOPHAN CONCENTRATION IN PLASMA BRAIN AND LIVER OF RABBITS

Control S. No.	Plasma (μg/mL)	Free (μg/mL)	Free (%)	Brain (μg/g)	Liver (μg/g)	Total liver protein (g/g)	Plasma glucose (g/mL)
1	9.92	4.76	48.00	6.04	12.34	0.356	218.32
2	10.70	3.32	31.00	5.59	18.13	0.246	245.25
3	10.66	5.95	55.81	5.28	18.37	0.272	300.69
4	7.20	2.99	21.14	5.10	12.17	0.249	352.44
5	6.80	3.20	22.63	4.92	13.32	0.200	300.00
Mean	9.05	4.04	35.71	5.38	14.86	0.26	283.34
SD	± 1.70	± 1.13	± 13.86	± 0.39	± 2.79	± 0.05	± 49.97
SE	± 0.76	± 0.51	± 6.21	± 0.17	± 1.25	± 0.02	± 21.06

A decrease in total tryptophan concentration in plasma, liver and brain is observed in rabbit. This decrease of total tryptophan concentration in plasma of apoenzyme lacking species suggests an enhanced tryptophan turnover *via* serotonin pathway[42].

In order to support this view the brain uptake of tryptophan in controls and ethanol treated animals is determined (Tables 2 and 3). The brain uptake is 48.27 % in control animals while the uptake is 59.44 % in ethanol treated animals supporting an enhanced tryptophan serotonin turnover in ethanol treated rabbits. Total protein concentration in liver and glucose level in plasma are not significantly changed in ethanol treated rabbits (Tables 2 and 3).

The results for total tryptophan concentration in plasma, brain and liver of control animals receiving tryptophan injection are given in (Table-4). The results indicate total and free tryptophan concentration is found to be 66.51 ± 4.29 and 43.24 ± 5.85 μg/mL in plasma, respectively. The total tryptophan concentration in brain and liver is found to be 72.59 ± 12.01 and 100.01 ± 23.65 μg/g, respectively.

TABLE-4
EFFECT OF TRYPTOPHAN LOADING IN CONTROL CHRONIC ETHANOL INDUCED CHANGES OF TRYPTOPHAN DISTRIBUTION IN PLASMA, BRAIN AND LIVER OF RABBITS

S. No.	Plasma (μg/mL)	Free (μg/mL)	Free (%)	Brain (μg/mL)	Liver (μg/mL)	Total liver protein (g/g)	Plasma glucose (g/mL)
1	80.64	52.99	65.71	108.41	191.17	0.240	313.86
2	69.62	46.08	66.66	44.29	44.29	0.240	235.03
3	70.26	60.56	86.19	54.69	112.90	0.243	445.74
4	53.30	29.27	54.91	101.60	100.05	0.243	397.20
5	58.75	27.33	46.51	54.00	51.67	0.320	300.00
Mean	66.51	43.24	63.99	72.59	100.01	0.25	338.36
SD	9.57	13.04	13.34	26.80	52.75	0.03	74.48
SE	4.29	5.85	5.98	12.01	23.65	0.01	33.40

Similarly, the results for total tryptophan concentration in plasma, brain and liver of ethanol treated rabbit receiving tryptophan injection are given in Table-5. The results indicate total and free tryptophan concentration is found to be 201.06 ± 18.48 and 118.56 ± 11.48 μg/mL in plasma, respectively.

TABLE-5
EFFECT OF TRYPTOPHAN LOADING ON CHRONIC ETHANOL INDUCED CHANGES OF TRYPTOPHAN DISTRIBUTION IN PLASMA, BRAIN AND LIVER OF RABBITS

S. No.	Plasma (μg/mL)	Free (μg/mL)	Free (%)	Brain (μg/mL)	Liver (μg/mL)	Total liver protein (g/g)	Plasma glucose (g/mL)
1	238.27	128.92	54.00	109.34	122.40	0.279	445.80
2	231.74	140.35	60.56	106.08	125.66	0.250	473.28
3	215.42	120.76	56.06	153.40	119.13	0.272	485.31
4	195.84	133.82	68.33	148.51	120.76	0.258	446.15
5	124.03	68.95	55.59	99.16	103.15	0.289	400.00
Mean	201.06	118.56	58.90	123.29	118.22	0.260	450.10
SD	± 41.21	± 25.61	± 5.19	± 22.87	± 7.83	± 0.010	± 29.39
SE	± 18.48	± 11.48	± 2.32	± 10.25	± 3.51	± 0.006	± 13.18

Furthermore, the total tryptophan concentration in brain and liver is found to 123.29 ± 10.25 and 118.22 ± 3.51 μg/g, respectively.

An intraperitoneal administration of tryptophan (500 mg/100 g body wt.) to control animals increases total tryptophan concentration by 302.35% in plasma and 552.80% in liver (Table-4).

In the ethanol treated animals tryptophan loading involved an increase total tryptophan concentration by 2121.65 % in plasma and 695.55 % in liver (Table-5). The results thus show a greater increase of tryptophan in plasma and liver of ethanol treated animals.

The increase in brain tryptophan concentration in control animals receiving tryptophan injection is 809.64 % while, it is 2191.63 % in ethanol treated animals receiving tryptophan injection (Table-5) suggesting an enhanced brain tryptophan metabolism in chronic ethanol treated rabbits[42].

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