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Short Term Effect of L-tryptophan Feeding on Blood Analytes and Cellular Na^+/K^+ ATPase Activity in Stressed Mice.

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ABSTRACT

Serotonin is an important neurotransmitter, derived from tryptophan. Normal serotonin levels are crucial for the organism's normal gut movements, mood and social behavior. Increased brain serotonin may improve the ability to cope with stress, while a decline in serotonin activity is involved in neuropsychiatric illnesses. Increasing dietary tryptophan (L-Trp) is known to improve brain serotonin levels. Na+, K+-ATPase is a stress indicator and its altered activity has been reported in mice exposed to various psycho-social stress. However, short term effects of L-Trp on ion transporters and stress response have not yet been identified in mice. In this study, we compared restrained and un-stressed mice for the short-term effects of dietary L-Trp on both blood metabolites as well as tissue Na+, K+-ATPase. Glucose, lactate and urea levels in plasma showed significant increase in the stressed mice ($p<0.001$), compared to normal mice. However, the levels declined in L-Trp-supplemented (2% L-Trp- enriched diet for 48 hours), stressed mice compared to untreated, stressed mice ($p< 0.01$). Neither restraint stress nor L-Trp supplementation affected blood pH, PaCO2, PaO2, tCO2, HCO3, Na^+, K^+ or Cl^- levels. Hepatic and renal Na^+/K^+-ATPase activity was elevated ($p< 0.01$) in stressed mice, compared to normal mice, which was significantly reduced by dietary L-Trp ($p< 0.01$). The data indicates that short-term tryptophan dietary supplementation can alleviate stress in mice.

Keywords: L-tryptophan, serotonin, stress, ATPases, feed, mice

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INTRODUCTION

Brain levels of serotonin are highly dependent on the levels of 5-hydroxytryptophan (5-HTP) in the central nervous system.[1] Biosynthesis of molecules having important cellular and neural functions including melatonin [2] require tryptophan. The synthesis of 5-HT from tryptophan occurs in several tissues throughout the body including the entero chromaffin (EC) cells of the gut, blood platelets and central nervous system.[3] The neurotransmitter 5-HT which is produced and active in nervous tissues and digestive tract is important for both the brain and the gastrointestinal tract (GIT) functions.[4,5] Oral intake of serotonin does not pass into the serotonergic pathways of the central nervous system since it is not permitted to blood–brain barrier. However, tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), from which serotonin is synthesized are allowed to cross the blood–brain barrier. The ratio of plasma tryptophan to the sum of the other large neutral amino acids is a factor that influence the cerebral availability of tryptophan (L-Trp -LNAA ratio).[6] Therefore, a tryptophan rich diet may increase brain serotonin synthesis and alter stressful implications.[6]

Since tryptophan is the precursor of serotonin, the amount of tryptophan in the diet can have important effects on the way the organism responds to stress. The present study presents the short-term effects of L-tryptophan administration on the ion transporter activity in restraint stress mice.

MATERIALS AND METHODS

The experimental animals were treated in accordance with Kerala University animal care protocol No.IAEC-KU-17/09-10-ZOO-MCSP (1). Healthy adult male Swiss albino mice (*Mus musculus*) born and reared in the laboratory were used in the study. Animals were housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. Animals weighing 28–35g were kept in polypropylene cages for mice (29 × 22 × 14 cm; length × width × height) with mesh – wire top. All animals were maintained on a lighting schedule (lights on at 7 a.m., lights off at 7 p.m.), and acclimated to our climate controlled animal facility at room temperature of 24 ± 4^{o}C and relative humidity of 70 ± 10% with minimum noise levels and less handling. Mice had ad libitum access to purified tap water and food (Sri Sai Durga Feeds and Products, Bangalore).

The experimental diets used were designed after the diet described by Iwalokun *etal*., 2006**. [7]** 2% Tryptophan enriched diet was prepared for supplementation. 150 g of standard feed (Sri Sai Durga Feeds and Products, Bangalore) was powdered and 3 g of L-Tryptophan (Sigma Aldrich) was added to it. The enriched diet was normal diet plus 2 g/100 g tryptophan.

Restraint stress: When needed, the mice were restrained according to the procedure described by Paula *et al*, 2007.[8] Animals were immobilized individually by using restraint tubes having length and diameter 12 cm and 5 cm respectively. The tubes with animals were placed vertically. This restraint procedure minimized the space around the animal, prevented its normal horizontal posture and its movements thus providing a rather strong, stressful stimulus without being harmful to the animal. This procedure resulted in an almost complete immobilization of the animals.

Treatment of animals: Sixteen mice were randomly assigned to one of four experimental groups assayed in parallel. The treatment regime was 48 hours. The first group (A) served as control. Controls were not given any kind of disturbance except for daily handling during the experimental period of 48 hours. The second group (B) was fed with 2% Tryptophan enriched diet. The third group (C) was subjected to restraint stress for 30 min daily (11.00 am-11.30 am). They were given normal standard diet. The fourth group was subjected to 30 min restraining for two days (11.00 am-11.30 am). They were given 2% Tryptophan enriched diet.

Sampling and analysis: After the 48 hours of treatment, the treated and control mice were lightly anaesthesized with chloroform (Sisco Research Labortaries, Mumbai, India). Blood samples were taken directly from the heart and analysed for blood gases. Heparin was used as anti-coagulant. Plasma was prepared by centrifugation at 5000 rpm for 10 min at 4°C for immediate use. The kidney and liver were collected and stored in 0.25 M SEI buffer (0.25 M sucrose, 10 mM EDTA, 0.1 M imidazole.) and stored at -80 °C for enzyme analysis.

Plasma Glucose:

Plasma glucose levels were determined colorimetrically using GOD/POD test kits. (Span Diagnostics Ltd., New Delhi). Glucose working solution was mixed with plasma samples and incubated at 37 ºC for 10 minute. After cooling at room temperature the samples were read at 505 nm with the help of a Double beam spectrophotometer (Systronics 2202, New Delhi).

Plasma Lactate:

Plasma lactate levels were estimated colorimetrically using standard method using lactate PAP fluid test kit (Radiant Diagnostics, Germany). Plasma samples were mixed with reaction mixture and incubated for 5 minute at room temperature. Quantification was done at 546 nm in a Double beam spectrophotometer (Systronics 2202, New Delhi).

Plasma Urea:

Plasma urea levels were estimated colorimetrically using standard method using DAM kit. (Span Diagnostics Ltd, New Delhi). Urea reagent mixed with plasma and DAM reagents were kept in a boiling water bath for 10 min, cooled in running tap water and the OD was measured at 525 nm using Double beam spectrophotometer (Systronics 2202, New Delhi).

Quantification of Na^+, K^+-ATPase specific activity:

A portion of the tissue homogenized in SEI buffer (pH 7.4) and centrifuged at 700 ×g for 10 minutes was used for analyzing the Na+, K+-ATPase activity. The ouabain-sensitive Na+, K+-ATPase-specific activity in the intestinal and liver tissue homogenates was quantified adopting the method of Peter et al. (2000).[9] Saponin (0.2 mg/mg protein) was routinely added to optimize substrate accessibility. The samples in duplicate were added to a 96-well microplate containing 100 mM NaCl, 30 mM immidazole (pH 7.4), 0.1 mM EDTA and 5 mM $MgCl_2$ with or without ouabain (2 mM) and incubated at 37 $^{\circ}$C. The reaction was initiated by the addition of ATP and was terminated with addition of 8.6% TCA. The liberated inorganic phosphate was determined in Autoreader 4011 (Spam Diagnostics Ltd., Surat, India) at 700 nm and expressed in µM Pi /h /mg protein.

Acid-base indices and minerals:

Blood pH, $PaCO_2$, PaO_2, tCO_2, HCO_3, $stHCO_3$ was measured with gas analyser (Opti CCA-TS; Opti Medical). Na^+, K^+ and Cl^- levels, haematocrit and haemoglobin in blood were measured using the ion analyser.

Data analysis:

Data were collected from four animals in each group. Statistical difference among groups were tested by means of one-way analysis of variance (ANOVA) followed by SNK comparison test. Significance between the groups were analyzed with the help of Graphpad Software (Graphpad Instat-3, San Diego) and the level of significance was accepted if $p < 0.05$.

RESULTS

We tested whether L-Trp has any role in counteracting the physiological effects produced by restraint stress. For this the mice were subjected to restraint stress for half an hour daily for two days. Half of the mice each from restrained as well as un-stressed groups were fed with a 2 % L-Trp-enriched diet.

Effect on plasma metabolites

The plasma glucose levels showed significant increase in the stressed mice (*P<0.001*) when compared to normal mice (Fig.1). However, the glucose levels declined (*P< 0.01*) in tryptophan supplemented stressed mice compared to untreated, stressed mice. The plasma lactate levels in the stressed mice showed an up-regulation (*P<0.001*) when compared to normal mice (Fig. 1). Lactate levels were down-regulated (*P< 0.01*) in

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stressed mice when supplemented with tryptophan. The plasma urea levels increased (*P<0.001*) in the stressed mice when compared to normal mice (Fig.1). The magnitude of urea level declined (*P< 0.01*) in tryptophan stressed mice when compared to stressed mice.

Effect on Na^+/K^+-ATPase activity

Hepatic Na^+/K^+-ATPase activity levels were elevated (*P*< 0.01) in stressed mice when compared to normal mice (Fig.2). Stressed mice with tryptophan supplementation showed a reduction (*P*< 0.01) in Na^+/K^+-ATPase activity in hepatic tissue when compared to stressed mice (Fig.2). Na^+/K^+-ATPase activity in renal tissues increased (*P*< 0.01) in stressed mice when compared to normal mice. The renal Na^+/K^+-ATPase activity showed a decline (*P*< 0.01) in tryptophan supplemented stressed mice when compared to stressed mice (Fig.2).

Acid-base indices and minerals

The treatment had no effect on tested acid-base indices. Neither restraint stress nor tryptophan supplementation affected blood pH, PCO_2, PO_2, tCO_2, HCO_3 levels (Table.1). Blood mineral status, [Na], [K] and [Cl], also remained unaffected after short term restraint stress or tryptophan supplementation (Table.2).

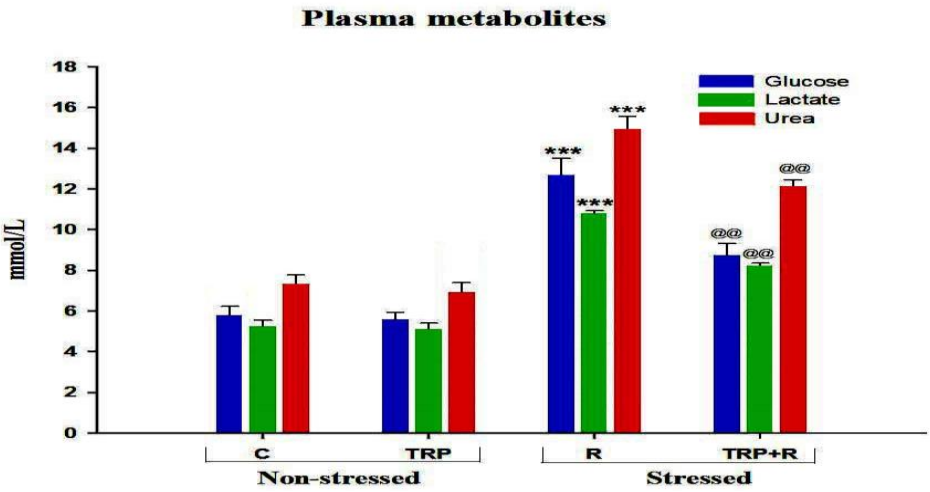

Fig 1: Plasma metabolites in mice treated with either tryptophan (L-TRP) or restraint (R). Each column represents mean ±SEM for five mice. *** P<0.001 denotes significant difference from control. $^{@@}P$< 0.01 denotes significant difference of L-TRP (2%)+ restraint mice from restraint mice.

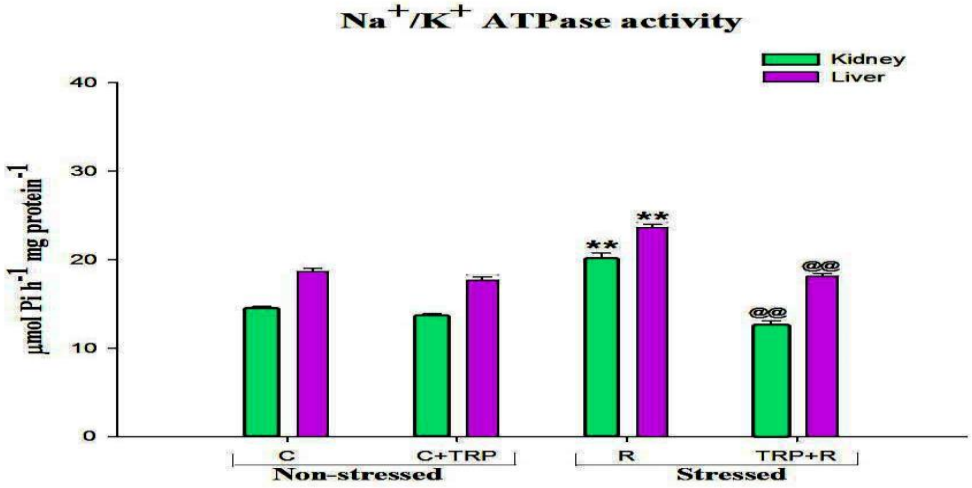

Fig 2: Na^+/K^+- ATPase activity in kidney and liver of mice treated with either tryptophan (L-TRP) or restraint (R). Each column represents mean ±SEM for five mice. **P< 0.01 denotes significant difference from control, $^{@@}P$< 0.01 denotes significant difference of L-Trp (2%)+60 min restrain from control+60 min restrain

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Table 1: Blood Na^+, K^+ and Cl^- levels (mmol/L) in un-stressed/restraint mice, treated/untreated with tryptophan.

	Status	Na^+	K^+	Cl^-
Un-stressed	**Control**	136.7±2.6	7.3±0.1	104.3±1.2
	Tryptophan	138.0±1.0	6.8±0.52	104.0±1.53
Stressed	**Restraint**	136.7±2.19	8.33±0.18	104.67±1.45
	Tryptophan+ restraint	143.0±1.53	8.3±0.2	107.17±0.88

Table 2: Blood indices in un-stressed/restraint mice, treated/untreated with tryptophan.

	Stressed		**Un-stressed**	
Status	**Control**	**Tryptophan**	**Restraint stress**	**Tryptophan+ Restraint stress**
pH	7.01±0.05	7.11±0.02	7.15±0.02	7.02±0.01
PaO_2	46.33±1.86	43.67±0.033	40.25±3.77	53±2.65
$PaCO_2$	89±7.32	73.33±4.33	87.67±4.63	64.67±0.88
tCO_2	24.23±0.86	25.33±0.63	24.3±0.29	23±0.38
HCO_3	21.6±1	22.93±0.54	20.75±0.89	21.1±0.12

DISCUSSION

A key aspect of the physiological stress response is the mobilization and re-allocation of energy substrates to meet the enhanced energy demand associated with stress. [10] Tryptophan increases brain serotonin concentrations [11] that have a profound influence during stress response. For example, 5-HT neurones fire at higher rates when an organism is aroused or stressed.[12] Likewise it has been postulated that tryptophan manipulations may exert a greater effect under arousing conditions [13], which include certain degree of provocation. The short-term effects of dietary tryptophan supplementation during restraint stress were studied in mice. It was found that the dietary intake of tryptophan can modify the tested variables.

Increased brain serotonin may improve the ability to cope with stress, whereas a decline in serotonin activity is involved in depressive mood.[14] A diet-induced increase in tryptophan availability may increase brain serotonin synthesis and improve coping and mood, particularly in stress-vulnerable subjects.[6] Increasing dietary trptophan causes increases in plasma and brain tryptophan and brain serotonin[15] and the transport of L-Trp over the blood–brain barrier depends on the concentration of L-Trp and LNAA.[16]

Energy supply is a limiting factor for physiology processes. The brain coordinates and integrates complex metabolic processes across various tissues. This ensures that appropriate nutrients reach individual organs, including the brain, in times of need. To do so, the central nervous system must be able to supervise fuel availability both directly and indirectly and to regulate not only food intake and body weight but nutrient supply, including blood glucose levels. The timely additional energy is required particularly in a stressful situation to which an organism responds by synthesizing and activating related enzymes, transporters, and enzymes. Glycogen metabolism is the principal energy source in both vertebrates and invertebrates, especially during responses to environmental fluctuations and stress.[17]

Analysis of plasma metabolic indices in our work revealed a hyperglycemic effect in mice kept for restraint. However supplementation of L-tryptophan in the diet gradually decreased the plasma glucose level. Plasma glucose has been considered as a very sensitive biomarker of stress and that indicates the magnitude of stress response.[18] The glucose production during stress helps the animal to cope with the increased energy demand. Role of dietary L-tryptophan as a stress mitigater was assessed in terms of reduced blood glucose was previously reported in the fish *Cirrhinus mrigala*.[19]

Lactate was also found to be another energy substrate of cells, especially in the central nervous system and myofibrils.[20, 21] During moderate activities, the lactate level doesn't grow significantly and can be metabolized quickly. But at higher effort levels, the lactate production increases much more. Stress too, whether physical or psychological in origin, elevates lactate levels.[22] The short-term treatment in our work showed a raised level of lactate in the restraint group. But L- tryptophan supplementation could counteract its the effect in stressed mice.

Plasma urea, the waste product of protein metabolism, in the blood is cleared from the bloodstream by the kidneys is an indicator to test renal function. An elevation in plasma urea may be caused by impaired renal function, dehydration, shock, acute myocardial infarction or stress. A rise in plasma urea in stressed mice indicates the response of mice towards restraint stress as L-tryptophan supplemention could lower its level in restraint mice. In mammals, Na^+/K^+-ATPase activity is responsible for a large part of the basic metabolic reactions and is also responsible for regulation of membrane polarization [23] and thermogenesis. Several factors have been identified that regulate Na^+/K^+- ATPase activity. Apart from allowing cells to deal with the sodium stress, Na^+/K^+- ATPase gave rise to an even higher level of sophistication by guiding hierarchic cellular functions such as motility, polarity, and signaling. It has been proved that Na^+-K^+- ATPase is a signal transducer.[24] Most of the effects have been studied in the kidney, where the physiologic regulation of Na^+/K^+- ATPase function has been well established. Na^+-K^+- ATPase activity in renal and hepatic tissues showed a significant rise in restraint mice. This indicates that Na^+-K^+- ATPase activity in these tissues may potentially be a response to increased Na^+ permeability in response to restraint stress, since elevated metabolic rate has been associated with increased leakiness in cell membranes. Thus, the elevation in Na^+-K^+- ATPase activity observed in the current study may be associated with the maintenance of normal transmembrane ion concentrations. Tryptophan supplementation of stressed mice showed a downregulation of Na^+-K^+- ATPase activity when compared to its activity in normal mice. Overall, our results suggest that short-term tryptophan supplementation through diet can exert a role in the stress mitigation in mice.

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REFERENCES

[1] Winberg S, Nilsson A, Hylland P. Neurosce Lett 1997; 230: 113–116.
[2] Maitra SK, Chattoraj A, Mukherjee S, Moniruzzaman M. Gen Comp Endocrinol 2012; 181: 215-222.
[3] Peters JC. Tryptophan nutrition and metabolism: an overview In: Schwarcz R (ed) Kynurenine and serotonin pathways. Plenum Press, New York 1991; 345–358.
[4] Vander A, Sherman J, Luciano D. Human physiology, the mechanisms of body function. Mc Graw Hill 1998; 207.
[5] Pilot MA, Thompson HH, Zara GP. J Physiol 1983; 343: 88–99.
[6] Markus R. Am J Clin Nutr 2000;71:1536–44.
[7] Iwalokun BA, Uwah A, Gbadegeshin Y, Matty F, Badaru O, Ariyo F. Afri J Biotech 2006; 5:1572-1578.
[8] Paula J, Matthias S, Georg W, Ulrike S, Hans-Guenther K, John AR, Peter R, Michael J. Endocrinolo 2007; 148(11): 5496–5506.
[9] Peter MCS, Lock RAC, Wendelaar BSE. Gen Comp Endocrinol 2000; 120: 157–167.
[10] Mommsen TP, Vijayan MM, Moon TW. Rev Fish Biol Fish 1999; 9: 211-268.
[11] Henry Y, Se`ve B, Colle`aux Y, Ganier P, Saligaut C, Je´go P. J Anim Sci 1992; 70: 1873–1887.
[12] Jacobs BL, Fornal CA. Neuropsychopharmacol 1999; 21: 9–15.
[13] Young SN, Pihl RO, Ervin FR. Clini Neuropharm 1988; 11: 207–215.
[14] Graeff FG, Guimaraes FS, de Andrade, TGCS, Deakin JFW. Pharmacol Biochem Behav 1996; 54: 129–41
[15] Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science (Washington, DC) 1971; 173: 149-151.
[16] Fernstrom JD. Physiol Rev 1983; 63: 484–546.
[17] Bacca H, Huvet A, Fabioux C, Daniel JY, Delaporte M, Pouvreau S, VanWormhoudt A, Moal J. Comp Biochem Physiol 2005; 140: 635–646.
[18] Peter VS, Peter MCS. J Endocrinol. Reprod 2007; 11: 8-14.
[19] Tejpal CS, Pal AK, Sahu NP. Aquaculture 2009; 293: 272–277.
[20] Porras OH, Loaiza A, Barros LF. J Neurosci 2004; 24: 9669-9673.
[21] Bergersen LH. Neurosci 2007; 145: 11-19.
[22] Uehara T, Sumiyoshi T, Matsuoka T, Tanaka K, Tsunoda M, Itoh H, Kurachi M. Brain Res Epub 2005; 1065: 86-91.
[23] Guerri C, Ribelles M, Grisolía S. Biochem Pharmacol 1981; 30(1):25-30.
[24] Xie Z, Askari A. Eur J Biochem 2002; 269: 2434-2439.