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Long-term tryptophan administration enhances cognitive performance and increases 5HT metabolism in the hippocampus of female rats

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Summary. It has been shown in various studies that increase in serotonergic neurotransmission is associated with increased memory consolidation whereas low brain 5HT impairs memory performance. In the first phase of our study we found that tryptophan (TRP) administration for 6 weeks increased plasma TRP and whole brain TRP, 5HT and 5HIAA levels. Many brain regions are involved in the learning process but particularly the hippocampus is known to have key role in learning and memory.

The present study was therefore designed to investigate the effects of TRP loading particularly on hippocampal 5HT metabolism and cognitive performance in rats. TRP-treated rats demonstrated spatial enhancement as evidenced by a significant decrease in time to find the hidden food reward in radial arm maze test (RAM). The important finding of the present study was the greater increase in the 5HT metabolism in hippocampus than in any other brain region of the TRP-treated rats. This increased 5HT metabolism in the hippocampus emphasizes the involvement of this region in memory process.

Keywords: Tryptophan – Memory – Hippocampus – Radial arm maze

Introduction

Tryptophan is an essential amino acid, the source of which is dietary only. It is the precursor of neurotransmitter serotonin. Brain serotonin synthesis depends on the uptake of its precursor tryptophan (TRP), which in turn is dependent on the plasma ratio of TRP to large neutral amino acids (LNAA) which compete for the same transport system in the brain (Feurte et al., 2001). TRP loading increases plasma TRP/LNAA ratio and increases brain 5HT and 5HIAA levels and therefore increases brain serotonin activity (Markus et al., 2002).

Serotonin system is widely distributed throughout the central nervous system. The existence of specific pathway projection from raphe nuclei to the forebrain and the density of 5HT receptors in these and other areas such as hippocampus and cortex support the growing body of evidence implicating 5HT in the process of learning and memory (Buhot, 1997; Buhot et al., 2000). It has been shown in various studies that increase in serotonergic neurotransmission is associated with increased memory consolidation (Harmer et al., 2002) whereas low brain 5HT impairs memory performance in humans (Hughes et al., 2002; Porter et al., 2003) and animals (Lieben et al., 2004).

Hippocampus is conceived as a key structure involved in long term spatial and working memory (Nadel and Moscovitch, 2001). Based on the above considerations, the present study was designed to investigate the effects of TRP administration on memory function and its relation with hippocampus in female rats.

Materials and methods

Subjects

Twelve locally-bred albino Wister rats (120–130 g) purchased from Agha Khan University Hospital were used in the study. All animals were housed individually under a 12 h light–dark cycle (light on at 6:00 h) and controlled room temperature (22 ± 2°C) with free access to cubes of standard rodent diet and tap water for at least 3–4 days before experimentation so that rats could adapt themselves to the new environment. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

Drug and treatment regimen

Tryptophan at a dose of 100 mg/kg/2 ml was administered orally to rats daily for 6 weeks. For oral administration a small stainless steel feeding tube fixed to a 1 ml syringe was used. The drug was carefully given in the mouth of rat and it was made sure that the rat took in the entire dose. Animals were randomly divided into control and test group. Test group received 100 mg/kg body weight TRP daily for 6 weeks. Weighed amount

of food was placed in the hopper of all the cages. Body weights and food intakes were monitored weekly. Behavioral activities of rats were also monitored. Rats were decapitated after 6 weeks between 10:00 and 11:00 h to collect plasma and brain samples. The experiment was performed in a balanced design in such a way that control and drug treated rats were killed alternately to avoid the order effect. After decapitation blood was collected in heparinized tubes and centrifuged to get plasma. These plasma samples were then stored below −70°C for estimation of TRP. Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline (0.9% w/v); hippocampus, cortex and rest of the brain were isolated and stored at low temperature (−70°C) until analysis of 5HT, 5HIAA and TRP by HPLC-EC.

HPLC-EC determination was carried out as standard (Haleem et al., 2002). A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), Octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure of 2000–3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu LEC 6A detector at an operating potential of 0.8 volts for biogenic amines and 1.0 volts for TRP.

Radial maze testing

Testing of cognitive function was performed in an eight arm radial maze. This procedure tests spatial working memory and assesses the integrity of the frontal cortex and hippocampus. The method used was a modification of method used by Neese et al. (2004). The maze utilized in this research study consisted of central platform (32 cm in diameter), which served as a starting base communicates with 8 arms of equal length (58 cm) and width (12 cm) distributed radially and each arm with 38 cm high plastic walls. 5 cm from the end of each arm a small plastic receptacle was placed to hold the food out of view from the center of the maze. The apparatus was mounted on a table so that it was 58 cm off the ground. We provided no special means to dispel the effect of smell because in radial maze vision is more important than smell. The maze was placed in laboratory where extramaze cues are there to facilitate learning. To ensure motivated performance rats were food-restricted to 50% of their daily food intake one week prior to RAM testing but had free access to water in the home cage.

RAM testing

RAM testing consisted of 3 phases:

Habituation
Rats were given 20 min habituation trial on 1st day with free access to all arms, one of which was baited with a hidden food reward. During habituation trial, rat freely visits each arm as many times as it likes. These were neither counted nor recorded.

Training
7 arms of the maze were blocked allowing access to only one arm. Food reward was placed at small intervals down the arm in order to entice the rat to the end of the arm. Once the rat reached the end of the arm, the rat was returned to the central platform and the arm was rebaited in order to continue the training procedure. Near the end of 15 min phase, food rewards were only placed at the end of the arm to train the rat to run to end of the arm to receive the reward.

Testing
The same arm that was baited with food during the training period was again baited with food during the testing session. The rest of the seven arms were also unblocked and rats had free access to all arms. Each rat was given 5 min to enter the arm, which was baited with food reward. The time elapsed before the rat entered the correct (baited) arm and the errors made by the rats were recorded in testing session. Each entry into any of the 7 unbaited arms with all four paws was counted as an error.

Statistics

Data are presented as mean ± SD. Neurochemical and behavioral data were analyzed by Student's *t*-test; p values <0.05 were considered significant.

Results

Influence of L-tryptophan upon weekly food intake and growth rate

Figure 1 shows that administration of 100 mg/kg TRP for 6 weeks did not alter the food intake or growth rate of rats.

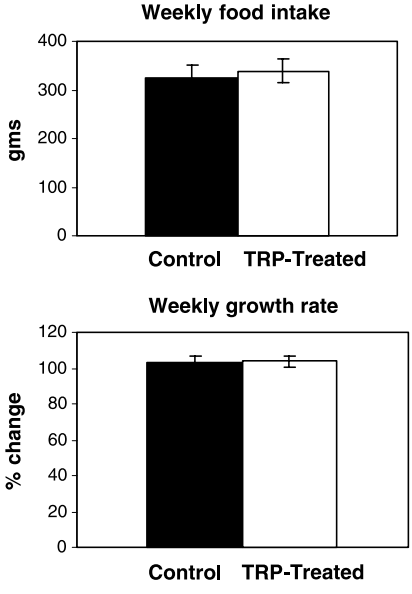

Fig. 1. Cumulative weekly food intake and growth rate in control and TRP-treated rats. Analysis by Student's *t*-test revealed no difference

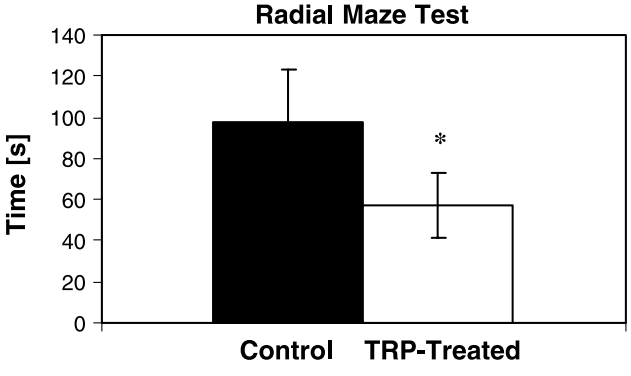

Fig. 2. Radial maze test in control and TRP-treated rats. Values are mean ± SD (n = 6). Significant differences by Student's *t*-test; *p < 0.01 vs. control rats

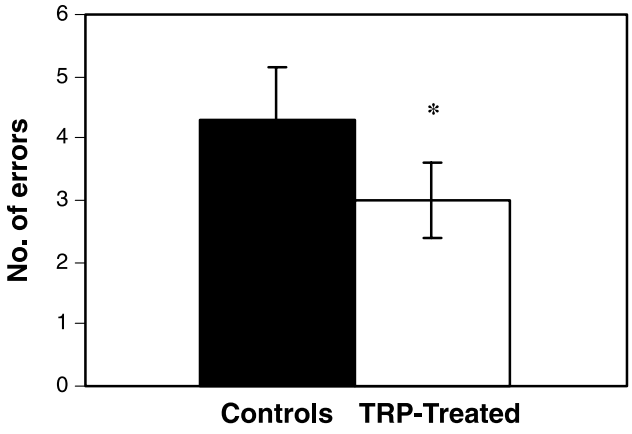

Fig. 3. Errors made by control and TRP-treated rats during 5 min test session in RAM. Values are ± SD (n = 6). Significant difference by Student's *t*-test; *p < 0.05 vs. control rats

Influence of L-tryptophan upon memory in rats as assessed by radial maze test

Figure 2 shows the alteration of performance in rats produced by daily administration of TRP in spatial working memory task. Analysis by Student's *t*-test revealed a significant effect on working memory ($t = 3.4$, df = 10, $p < 0.01$). The results indicate that TRP administration enhances working memory functions.

Influence of L-tryptophan upon errors made by rats in radial maze test

Figure 3 shows the errors made by control and TRP treated rats during the 5 min test session. Analysis by Student's *t*-test revealed a significant decrease in errors ($t = 3.13$, df = 10, $p < 0.05$) made by TRP-treated rats.

Influence of L-tryptophan upon 5HT levels in various regions of brain

Figure 4 shows that administration of 100 mg/kg TRP for 6 weeks significantly increased 5HT concentration in hippocampus ($t = 8.57$, df = 10, $p < 0.01$), cortex ($t = 4.26$, df = 10, $p < 0.01$) and rest of the brain ($t = 3.65$, df = 10, $p < 0.01$). Percentage increase was greatest in the hippocampus as shown (Fig. 4).

Influence of L-tryptophan upon 5-HIAA levels in various regions of brain

Figure 5 shows the effect of TRP administration on brain 5HIAA levels. Analysis by Student's *t*-test revealed a significant increase in 5HIAA levels in hippocampus

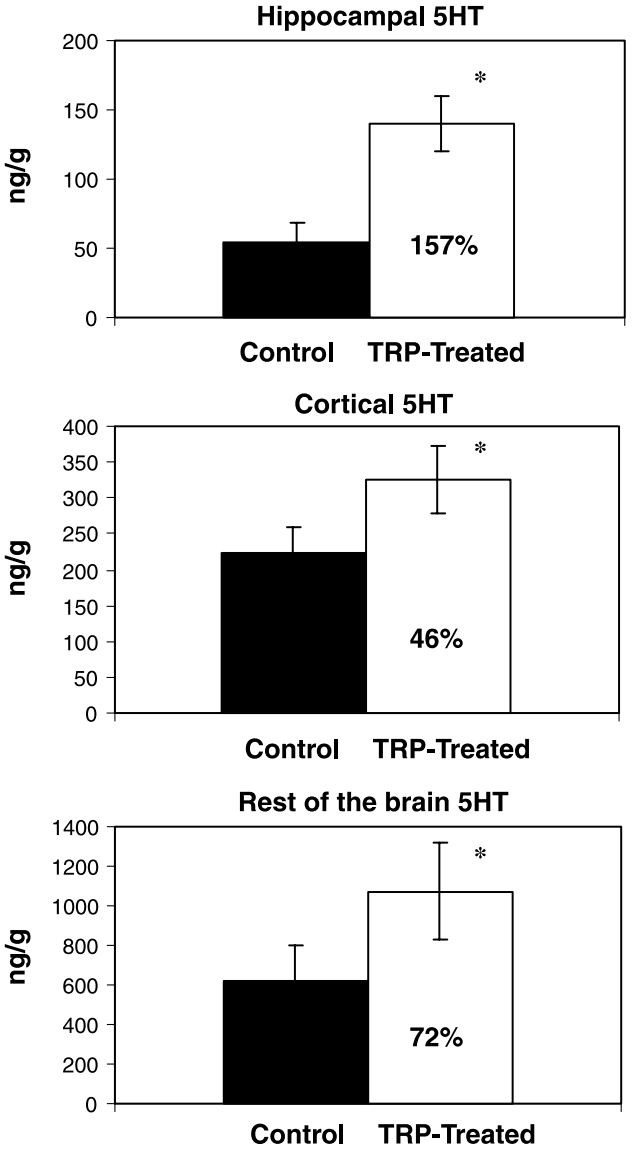

Fig. 4. Hippocampal, cortical and rest of the brain 5HT in control and TRP-treated rats. Values are mean ± SD (n = 6). Significant differences by Student's *t*-test; *p < 0.01 vs. control rats

($t = 8.58$, df = 10, $p < 0.01$), cortex ($t = 4.35$, df = 10, $p < 0.01$) and rest of the brain ($t = 2.97$, df = 10, $p < 0.05$). Percentage increase of 5HIAA was greatest in the hippocampus as shown (Fig. 5).

Influence of L-tryptophan upon plasma TRP levels and TRP in various regions of brain

Figure 6 shows the effect of TRP administration on plasma TRP and TRP in various regions of the brain. Analysis by Student's *t*-test revealed a significant increase in levels of TRP in plasma ($t = 3.99$, df = 10, $p < 0.01$), cortex ($t = 3.45$, df = 10, $p < 0.01$) and rest of the brain ($t = 2.9$, df = 10,

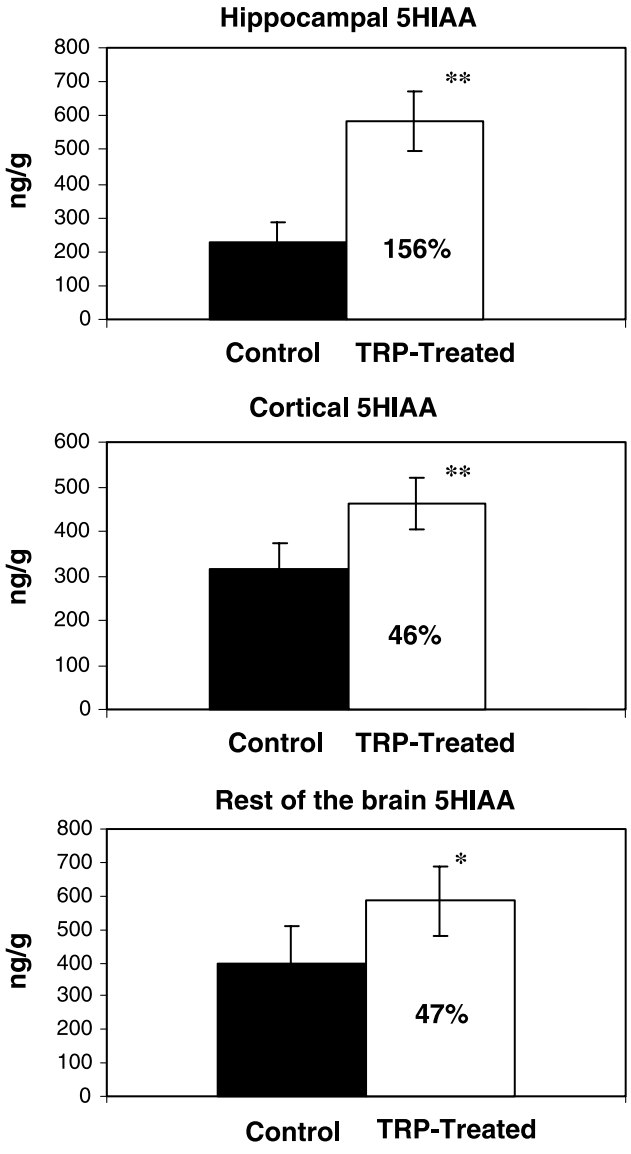

Fig. 5. Hippocampal, cortical and rest of the brain 5HIAA in control and TRP-treated rats. Values are mean ± SD (n = 6). Significant differences by Student's *t*-test; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control rats

$p < 0.05$). TRP levels in hippocampus were comparable which shows the greater conversion of TRP to 5HT.

Discussion

In the present study administration of tryptophan for 6 weeks increased serotonin metabolism in all isolated regions of the brain. The increase in 5HT metabolism was, however largest in the hippocampus than in any other region of brain. Increased levels of 5HT precursor tryptophan were found in plasma of TRP-treated than control rats. Tryptophan levels which were significantly increased in other brain regions were comparable in the hippocampus, indicating a greater conversion of tryptophan to 5HT in this region.

Serotonin synthesis depends on brain tryptophan levels, which in turn depends on blood tryptophan concentration. Elevated tryptophan in the brain produces physiologically important changes in serotonergic system. The precise role of the serotonergic system in cognition is complex. Among many other functions 5HT neurotransmission is known to play an important role in the mechanism of learning and memory (Essman, 1974). Actually, 5HT pathways, 5HT reuptake site/transporter complex and 5HT receptors show regional distribution in brain areas implicated in learning and memory (Meneses, 1999). Increased brain 5HT activity is suggested to improve cognitive performance (Markus et al., 2002), whereas decreasing brain 5HT levels has been shown to impair cognition (Riedel et al., 2003). However, there are also reports which indicate no effect on cognitive function with decreased 5HT levels following tryptophan depleted diet (Stancampiano et al., 1997). In the first phase of our study we found that tryptophan administration for 6 weeks increased plasma tryptophan and whole brain tryptophan, 5HT and 5-HIAA levels (Khaliq et al., 2006). Memory of rats assessed by radial maze and passive avoidance test was also found to be improved. The enhanced cognitive performance in rats was linked to the increased 5HT metabolism in brain. In the present study 5HT metabolism following tryptophan administration was particularly investigated in regions known to be involved in learning and memory processes. The hippocampus is a major limbic target of the brainstem serotonergic neurons that is known to modulate learning and memory. It has been extensively studied for its role in spatial working memory (Lee and Kesner, 2003). Many studies have demonstrated that 5HT plays an important role in hippocampal dependent learning (Meneses, 1999; Buhot et al., 2000; Nadel and Moscovitch, 2001). This is reflected in disorders, where low 5HT function has been implicated and long term memory deficits are common (McEntee and Crook, 1991; Richter-Levin and Segal, 1996; McAllister-Williams et al., 1998; Harrison, 2002).

In the current study, the effects of increased serotonin metabolism on cognitive function following tryptophan administration were examined by radial maze task. Cognitive performance was significantly enhanced in TRP-treated than control rats. In the radial maze compared to controls the TRP-treated rats made less errors and took less time to enter the correct (baited) arm. The important finding of the present study was a significant increase in 5HT metabolism particularly in the hippocampus of the brain, where involvement of learning and memory is well documented. Our findings therefore suggest that enhancement of spatial working memory by TRP administration might be related to increase in serotonergic metabolism in the hippocampus.

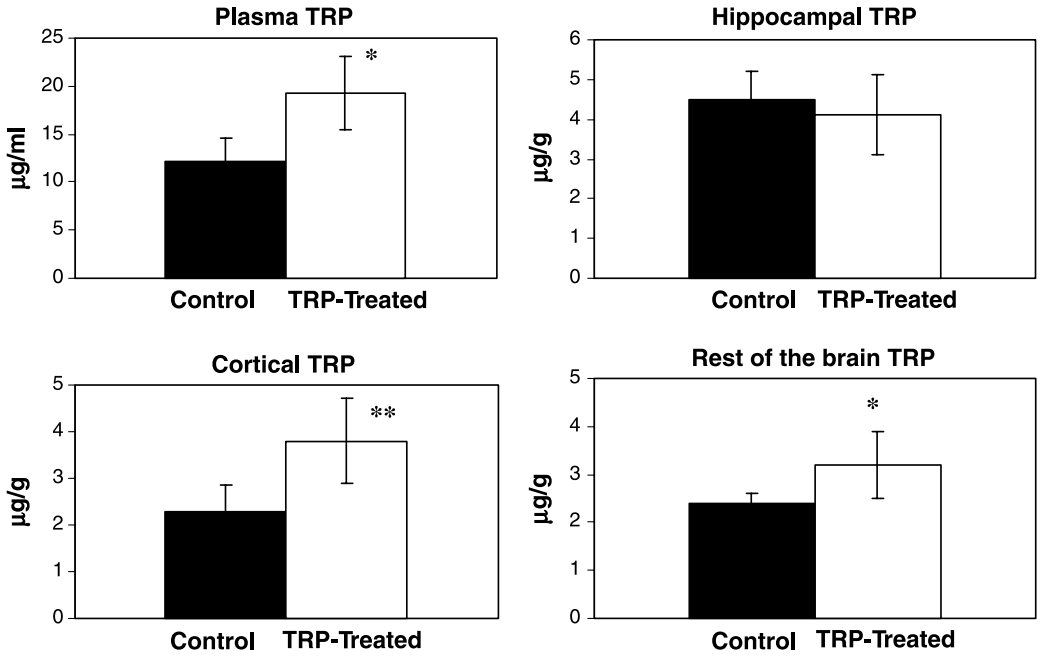

Fig. 6. Plasma tryptophan and hippocampal, cortical and rest of the brain TRP in control and TRP-treated rats. Values are mean ± SD (n = 6). Significant differences by Student's *t*-test; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control rats

Thus in conclusion, the increased 5HT metabolism in the hippocampus than in any other region of the brain of TRP-treated rats suggests the involvement of this region in the enhancement of memory function in rats.

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