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Enhanced serotonergic neurotransmission in the hippocampus following tryptophan administration improves learning acquisition and memory consolidation in rats

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Abstract:
Increasing evidence shows that serotonin (5-hydroxytryptamine – 5-HT) plays a modulatory role in memory functions. 5-HT transmission has been implicated in learning and memory. Both 5-HT depletion and specific 5-HT agonists lower memory performance. Hippocampus is thought to be the key region involved in long-term memory. It is the major limbic target of the brainstem serotonergic neurons that modulate learning. In the present study, we examined the effects of increased hippocampal 5-HT metabolism following tryptophan (TRP) administration on short-term memory (STM) and long-term memory (LTM) in rats. Learning acquisition (LA) and memory consolidation (MC) in rats was also evaluated. TRP at 50 mg/kg and 100 mg/kg body weight was used. Assessment of memory in rats was done using the water maze test (WM) after 6 weeks of daily administration of TRP. The results showed that administration of TRP enhanced both STM and LTM. However, the effect on STM was significant only at the higher dose. Rats administered the higher dose of TRP also exhibited a significant enhancement in LA. A significant effect on MC was also observed in tryptophan-treated rats. The results suggest that serotonergic system in the hippocampus is important in LA and MC in rats.

Key words:
tryptophan, 5-HT, hippocampus, learning acquisition, memory consolidation, water maze test

Introduction

Tryptophan (TRP), an essential amino acid, is the precursor of serotonin (5-HT) involved in a number of physiological functions. One of the important roles of 5-HT is in the regulation of memory processes [6, 7]. Increased brain 5-HT concentration has been shown to enhance cognitive function [8, 10] whereas decreased 5-HT metabolism in the brain has been shown to impair memory [2, 11, 15]. The role of the hippocampus in mediating cognitive function such as learning and memory is well established [9, 13]. Memory regulation is particularly associated with serotonergic system in the hippocampus [1, 12]. The learning process is integrated from sensorial inputs and is consolidated in short term memory (STM) or long-term memory (LTM). TRP and hence 5-HT is strongly related to both types of memory [19]. Studies into the role of 5-HT in cognitive functioning have provided

evidence on serotonergic involvement in learning and memory. In most of the previous studies, acute TRP depletion method has been used to study the serotonergic functions. We have previously shown that administration of TRP increased 5-HT metabolism in the hippocampus and improved the cognitive performance in rats [3].

The present work extends our evaluation of tryptophan's cognition enhancing potential by determining the effect of TRP administration on learning acquisition (LA) and memory consolidation (MC) in rats.

Materials and Methods

Animals

Eighteen locally bred albino Wistar rats (180–200 g) purchased from Aga 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. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

Drug administration

TRP at a dose of 50 mg/kg/2 ml and 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 into the mouth of rat and it was made sure that the rat took in the entire dose.

Experimental protocol

Animals were randomly divided into control and test groups. Weighed amount of food was placed in the hopper of all the cages. Body weight 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 experiments were performed in a balanced design in such a way that the control and drug-treated rats were killed alternatively 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 s of the decapitation. Hippocampus was immediately separated and stored at low temperature (–70°C) until analysis of 5-HT, 5 hydroxyindoleacetic acid (5-HIAA) and TRP by high performance liquid chromatography EC (HPLC-EC).

HPLC-EC determination was carried out according to standard procedure [4, 5]. 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 at pH 2.9 at an operating pressure of 2000–3000 psi on Schimadzu LEC 6A detector at an operating potential of 0.8 V for biogenic amines and 1.0 V for TRP.

Behavioral tests

Water maze test

The effects on spatial memory were examined by assessing performance in the water maze (WM) test developed in our laboratory [14]. The WM apparatus used in the present study consisted of a transparent rectangular glass tank (60 × 30 cm) filled with water at room temperature opacified with powder milk, tothe depth of 12 cm (Fig. 1). A wooden platform (15 × 13 cm) was hidden 2 cm below the surface of water in a fixed location. The experiment was performed 6 weeks after daily administration of TRP. Ini-

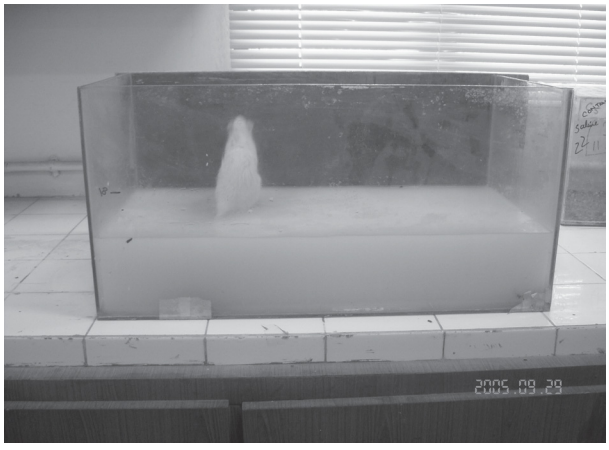

Fig. 1. The water maze apparatus used in the present study

tially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 s to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 s. If it failed to find the platform within the allowed time it was guided gently onto the platform. After the training animals were tested three times; first for acquisition; each rat was tested immediately after training. LA was determined by recording the initial latency (IL; the time taken by each rat to relocate the hidden platform). Second time rats were tested for STM 60 min after training and finally for LTM 24 h later. The STM and LTM were determined by recording the retention latency (RL; the time taken by each rat to locate the hidden platform 1 h and 24 h after training). The cut off time for each session was 2 min.

Statistical analysis

Results are presented as the mean ± SD. Neurochemical and behavioral data were analyzed by one-way ANOVA. *Post-hoc* analysis was done by Newman-Keuls test. Comparison for the effect of TRP on memory consolidation was done by Student's *t*-test; p value < 0.05 was considered significant.

Results

Effect of L-tryptophan upon 5-HT and 5-HIAA levels in the brain

Concentration of 5-HT and 5-HIAA were determined in the hippocampus following administration of 50 and 100 mg/kg TRP for 6 weeks. Analysis by one-way ANOVA revealed significant effect following TRP administration on 5-HT and 5-HIAA ($F = 41.9$, $p < 0.01$ and $F = 42.6$, $p < 0.01$, respectively). *Post-hoc* analysis by Newman-Keuls test showed that 5-HT and 5-HIAA levels were significantly increased at both 50 ($p < 0.01$) and 100 mg/kg ($p < 0.01$) doses of TRP (Fig. 2).

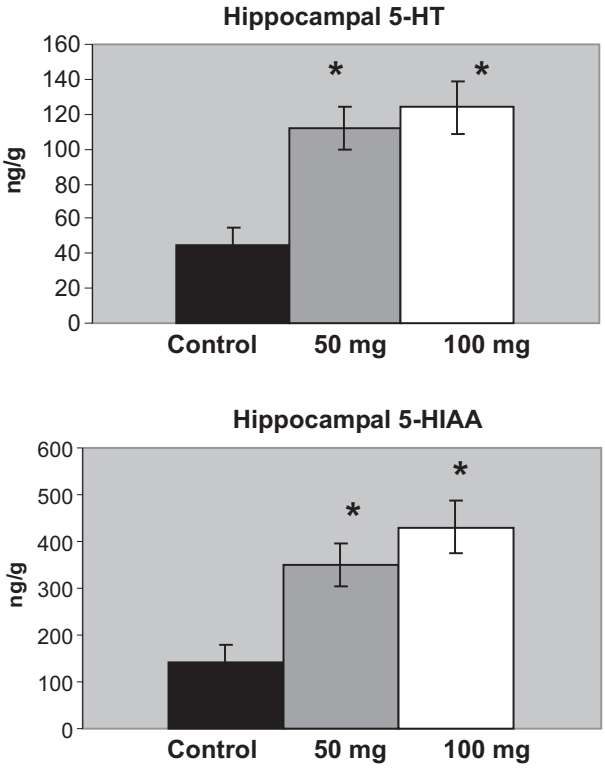

Fig. 2. Effect of TRP on hippocampal 5-HT and 5-HIAA. Values are the means ± SD (n = 6). Significant difference according to Newman-Keuls test following one-way ANOVA: * $p < 0.01$ *vs.* respective water-treated controls

Effect of L-tryptophan upon learning acquisition in rats as assessed by the water maze test

Effect of 50 mg and 100 mg/kg TRP on learning acquisition in rats was assessed immediately after the first exposure (training) of rats to WM. One-way ANOVA revealed a significant treatment effect ($F = 10.83$ $df = 2, 15$ $p < 0.01$). *Post-hoc* analysis showed that the latency time of rats given 100 mg/kg was significantly ($p < 0.01$, 34.6%) smaller compared to control rats whereas the latency time of rats given 50 mg/kg TRP was comparable to that of controls (Fig. 3).

Effect of L-tryptophan upon short-term memory in rats

Effect of TRP on STM was assessed one hour after the first trial. Analysis by one-way ANOVA showed a significant treatment effect ($F = 11.7$ $df = 2, 15$ $p < 0.01$). *Post-hoc* analysis showed that the STM was significantly ($p < 0.01$, 54%) improved following

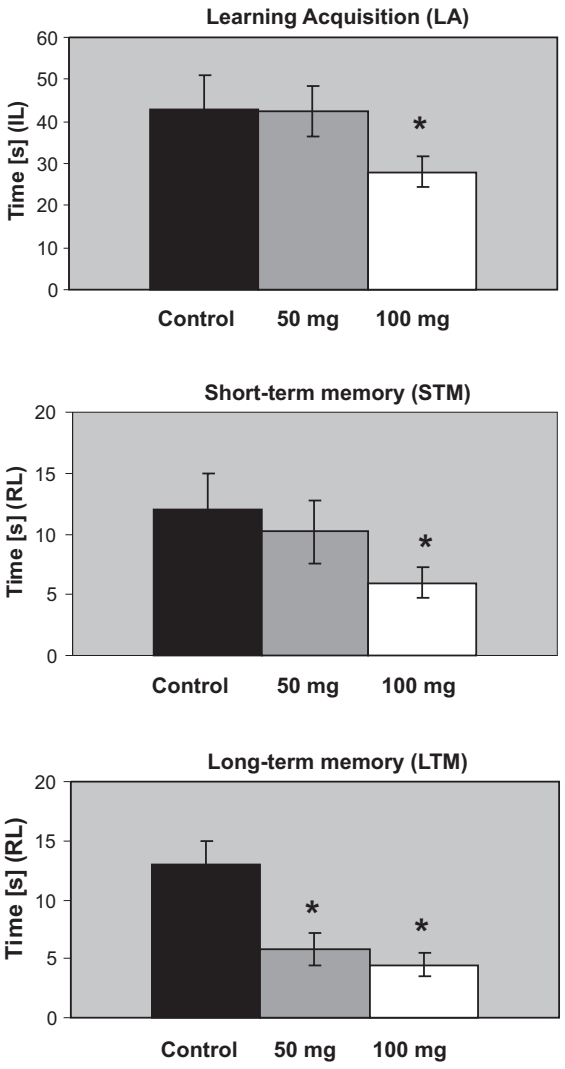

Fig. 3. Effect of TRP administration on LA, STM and LTM. Values are the means ± SD (n = 6). Significant difference according to Newman-Keuls test following one-way ANOVA: * $p < 0.01$ *vs.* respective water-treated controls. IL – initial latency, RL – retention latency

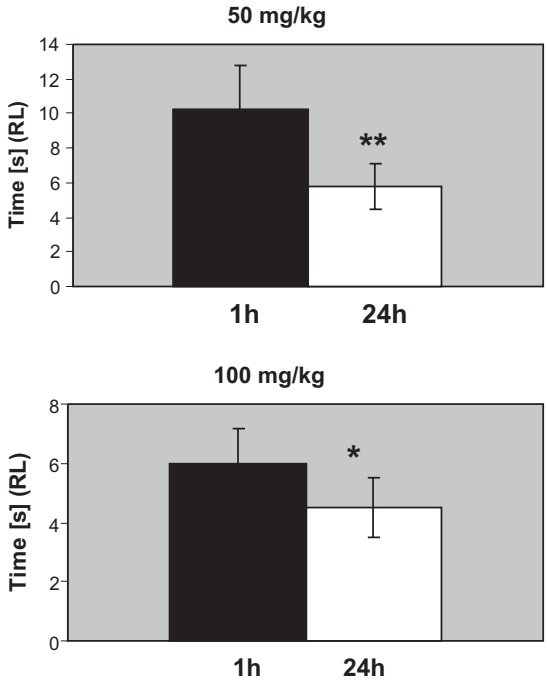

Fig. 4. Effect of TRP administration on memory consolidation. Values are the means ± SD (n = 6). Significant difference according to Student's *t*-test: * $p < 0.05$, ** $p < 0.01$ *vs.* 1 h control values following training in water maze test. RL – retention latency

100 mg/kg TRP, whereas rats given 50 mg/kg TRP did not show any effect on STM (Fig. 3).

Effect of L-tryptophan upon long-term memory in rats

LTM was assessed 24 h after the first trial. Analysis by one-way ANOVA showed a significant treatment effect ($F = 76.13$ df = 2, 15 $p < 0.01$). *Post-hoc* analysis showed that LTM was significantly improved following administration of both 50 mg ($p < 0.01$, 66.5%) and 100 mg/kg ($p < 0.01$, 72.4%) doses of TRP (Fig. 3).

Influence of L-tryptophan on memory consolidation in rats

MC effect of TRP was assessed by comparing the retention latency at the same dose 1 h and 24 h after training. The results showed a significant improvement in memory retention after 24 h. Analysis by Student's *t*-test revealed a significant decrease in RL at both 50 mg ($p < 0.01$) and 100 mg ($p < 0.05$) doses of TRP after 24 h (Fig. 4).

Discussion

The primary aim of the study was to investigate the effects of increased 5-HT synthesis in the hippocampus by TRP administration on memory functioning. It was hypothesized that administration of TRP would affect the cognitive function due to an increase in brain 5-HT levels. The present study shows that long-term TRP administration improves MC and has a positive effect on LA in rats.

The role of the hippocampus in mediating cognitive function is well established [9, 13]. Reduced serotonergic function in the hippocampus has been shown to contribute to age-related memory deficits

[16,18]. We have also previously shown an association between increased 5-HT metabolism in the hippocampus and enhanced cognitive performance in rats [3]. The present results are in agreement with our previous findings and findings of others [3, 9, 16]

A number of acute TRP depletion studies have previously reported an impairment in MC [17, 19]. The present work describes the effects of TRP administration on these parameters. The results of the study demonstrate that administration of TRP markedly increased LA and MC in rats. The increase in acquisition was observed at 100 mg/kg dose of TRP but not at 50 mg/kg. This increase in acquisition was evident from decreased IL exhibited by the rats given 100 mg/kg TRP, which shows that initial learning is improved by TRP but at a higher dose. Regarding the STM which was evaluated 1 h after training, the effect of TRP administration was again observed only at 100 mg/kg TRP dose. Rats given 100 mg/kg TRP exhibited enhanced memory function exhibiting decreased retention latency compared to controls. The retention latency in rats given 50 mg TRP was comparable to that of controls. However, 24 h later when the same rats were tested for LTM, an improvement in memory function was observed at both doses. Our finding of lack of any effect at 50 mg/kg after 1 h but improved memory function at the same dose after 24 h is an evidence of MC in TRP-treated rats. Further evidence of improved memory retention is provided by the fact that RL at 100 mg/kg was more decreased after 24 h which highlights the role of TRP in improving memory retention (MC) with the passage of time.

Together the neurochemical and behavioral data suggest that increase in serotonergic neurotransmission in the hippocampus plays an important role in improving LA and MC in rats. The results further indicate that administration of TRP as a dietary supplement may be useful for enhancement of memory functioning.

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