
ORIGINAL ARTICLE

**COMPARATIVE EFFECTS OF SINGLE DOSE AND REPEATED ORAL
TRYPTOPHAN ADMINISTRATION ON INDOLEAMINE SYNTHESIS
AND MEMORY FUNCTIONS IN RATS**

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ABSTRACT

Brain functions can be affected by the availability of dietary precursors of neurotransmitters. The diet induced increase in tryptophan (TRP) availability has been shown to increase brain serotonin (5-HT, 5-hydroxytryptamine) synthesis and various related behaviors. A prominent role of serotonin in memory functions is widely acknowledged. Increased brain 5-HT concentration is shown to enhance cognitive function whereas decreased 5-HT metabolism in brain has been shown to impair memory. This study was designed to investigate

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the effects of single dose and repeated TRP administration on brain TRP, 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) levels and on memory functions in rats. TRP at a dose of 100 mg/kg body weight were orally administered to rats. Assessment of memory in rats was done using the water maze test (WM). Brain TRP, 5-HT and 5-HIAA levels were comparable to control after single TRP administration. Repeated administration of TRP for 6 weeks significantly increased brain TRP ($P<0.05$), 5-HT ($P<0.01$) and 5-HIAA ($P<0.01$) levels with respect to controls. Memory enhancement effect of TRP was not seen after single oral administration whereas repeated TRP intake significantly ($P<0.01$) enhanced memory functions of rats as evidenced by the decreased latency time to reach the hidden platform in WM. Our results indicate that repeated but not single oral TRP administration is involved in the enhanced memory functions in rats.

Keywords: tryptophan, 5-HT, memory, water maze test.

INTRODUCTION

TRP 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 TRP (Curzon, 1981), which in turn is dependent on the plasma ratio of TRP to large neutral amino acids (LNAAs) which compete for the same transport system in brain (Feurte *et al.*, 2001). TRP loading increases plasma TRP/LNAAs ratio and increases brain TRP, 5-HT and 5-HIAA levels and therefore increases brain serotonin activity (Markus *et al.*, 2002).

TRP is widely used as a natural tool for the support of serotonin mediated brain functions and as a challenge probe for the serotonin mediated behavioral responses. In normal circumstances brain TRP is an important factor for controlling the synthesis of 5-HT (Fernstrom, 1985). TRP hydroxylase, the rate limiting enzyme of the biosynthesis is only 50% saturated with its substrate (Hamon *et al.*, 1981; Young and Gauthier, 1981) therefore an increase in TRP will tend to automatically increase brain serotonin production (Young and Teff, 1989). It has been well established that increased serotonergic neurotransmission is associated with increased memory consolidation (Laercio *et al.*, 2004; Haider *et al.*, 2006) whereas low brain 5-HT impairs memory functions (Porter *et al.*, 2003; Haider *et al.*, 2005). Previous studies showed that single TRP injection did not alter 5HT and 5HIAA levels (Bergqvist *et al.*, 1996) while other studies reported that TRP administration increased 5HT synthesis (Haleem *et al.*, 1998).

We have previously reported that increased 5-HT synthesis by repeated TRP administration for 6 weeks enhanced memory functions (Khaliq *et al.*, 2006). The present study is designed to specifically investigate the effects of single and repeated TRP administration on brain TRP, 5-HT and 5-HIAA levels and on memory functions in rats.

MATERIALS AND METHODS

Animals

Eighteen adult male locally bred albino Wister rats (180-200g) 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 6:00h) and controlled room temperature ($22 \pm 2^{\circ}\text{C}$) 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.

Chemicals

TRP was purchased from Merck (Germany). The methanol used in the study was obtained from Sigma Chemicals (USA). All the other chemicals used were also of the highest quality available.

Drug administration

TRP at a dose of 100mg/kg body weight was orally administered to rats. 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. An equal amount of vehicle (water) was given orally to control rats.

Experimental protocol

Animals were randomly divided into control and two test groups. One test group received 100 mg/kg TRP for 6 weeks to determine the effect of repeated TRP administration while the other group received an equal dose of TRP orally only on the last day to determine the effect of single dose of TRP. 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 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 brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation and stored at low temperature (-70°C) until analysis of 5-HT, 5-HIAA 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

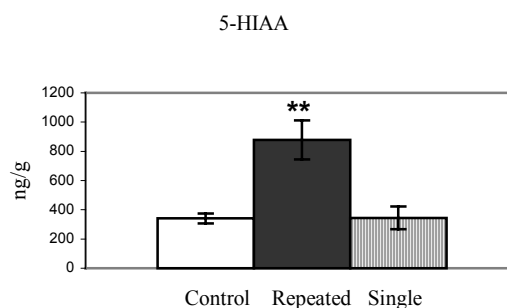
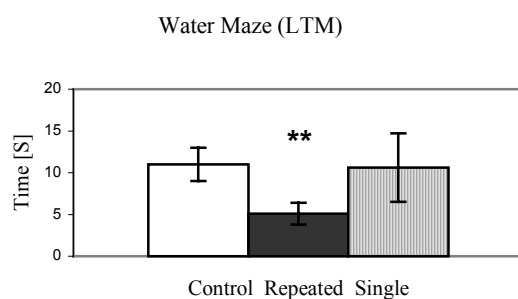
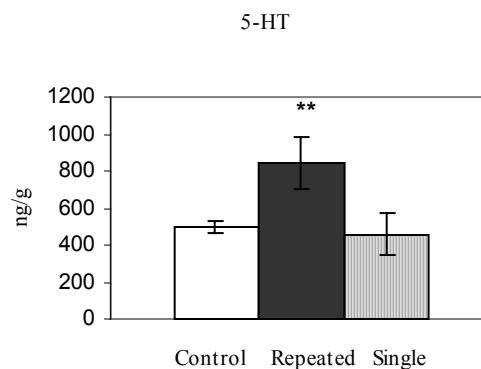
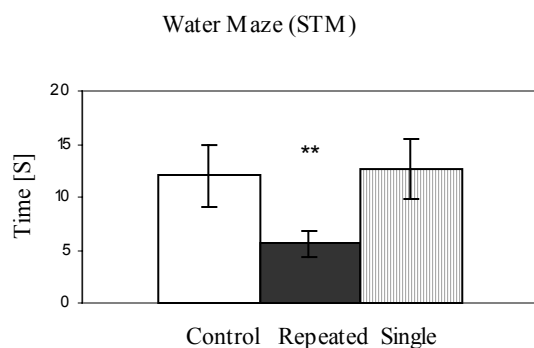
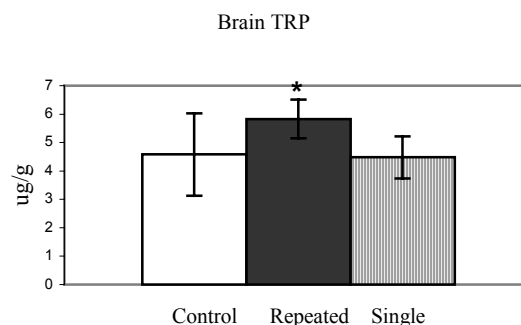
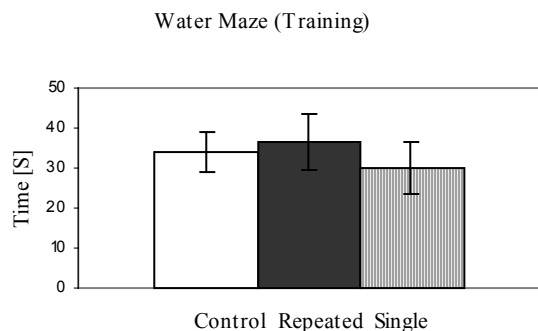


Fig. 1. Effect of single and repeated L-tryptophan administration on short term memory and long term memory in water maze test. Values are means $\pm$ S.D (n = 6). Significant difference by Neuman-Keuls test. Following one- way ANOVA. **p<0.01 from respective water treated controls.

Fig 2. Effect of single and repeated L-tryptophan administration upon plasma brain tryptophan, 5-hydroxytryptophan and 5-hydroxyindole acetic acid levels. Values are means $\pm$ S.D (n = 6). Significant difference by Neuman-Keuls test. Following one- way ANOVA. *p<0.05; **p<0.01 from respective water treated controls.

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 2000-3000psi on Shimadzu LEC 6A detector at an operating potential of 0.8 volts for biogenic amines and 1.0 volts for TRP.

Behavioral test

Water Maze Test

The effects of single dose and repeated TRP administration on spatial memory was examined by

assessing performance in a Water Maze test developed in our laboratory (Khaliq *et al.*, 2006). The WM apparatus used in the present study consisted of a transparent rectangular glass tank (60 x 30cms) filled with room temperature-water opacified with powder milk, to the depth of 12cm. A wooden platform (15 x 13cms) was hidden 2cm below the surface of water in a fixed location. The experiment was performed after 6 weeks for examining the effect of repeated TRP administration on memory. Initially 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 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 seconds. If it failed to find the platform within the allowed time it was guided gently onto the platform. Animals were tested for short term memory (STM) 60 minutes after training and finally for long term memory 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 1h and 24 h after training). The cut off time for each session was 2 minutes.

STATISTICAL ANALYSIS

Results are presented as mean $\pm$ SD. Neurochemical and behavioral data were analyzed by one way ANOVA. Posthoc analysis was done by Newman-Keuls test. P values < 0.05 were considered significant.

RESULTS

Effect of single and repeated L-Tryptophan administration 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 ($F=15.1$ df 2,15 $P<0.01$). Post hoc analysis showed that STM was significantly improved following repeated TRP administration ($P<0.01$). No effect on STM was observed following single administration of TRP (fig. 1b).

Effect of single and repeated L-Tryptophan administration upon long term memory in rats

Long term memory was assessed 24 hour after the 1st trial. Analysis by one way ANOVA showed a significant treatment effect ($F=8.04$ df 2,15 $P<0.01$). Post hoc analysis showed that LTM was significantly improved following repeated TRP administration ($P<0.01$). No effect on LTM was observed following single administration of TRP (fig. 1c).

Effect of single and repeated L-Tryptophan administration upon TRP, 5HT and 5HIAA levels in brain

Concentration of brain TRP, 5HT and 5HIAA were determined following single and repeated administration of 100mg/kg TRP. Analysis by one way ANOVA revealed significant effect following TRP administration on brain TRP ($F=4.9$ df 2,15 $P<0.05$), 5HT ($F=24.3$ df 2,15 $P<0.01$) and 5HIAA ($F=67.8$ df 2,15 $P<0.01$). Post hoc analysis by Neuman Keuls showed that TRP ($P<0.05$), 5HT ($P<0.01$) and 5HIAA ($P<0.01$) levels were significantly increased after repeated TRP administration whereas single oral administration of TRP did not increase 5HT levels with respect to controls (fig. 2).

DISCUSSION

One manner to study the role of 5-HT in behavioral functions is through nutritional manipulation of its precursor TRP. It is an essential amino acid and its source is solely dietary. In the present study we examined the effect of single and repeated oral administration of TRP at a dose of 100 mg/kg on the alteration in learning and memory performance and 5-HT metabolism in rat brain. Two hours after single administration of TRP, rats were trained in WM. STM and LTM were then assessed after 1h and 24h respectively. Rats administered with the single dose of TRP showed no difference in retention latency with respect to controls. Other group of rats fed with the same dose of TRP repeatedly for 6 weeks showed an increase in memory performance in WM as evidenced by decrease in latency time to reach the platform. The repeated oral administration of TRP produced a significant increase in brain TRP, 5-HT and 5-HIAA levels compared to controls whereas single oral administration of TRP did not show any increase in 5-HT metabolism.

Previous study showed that alteration in brain serotonin levels were also associated with altered levels of its precursor TRP in the brain (Weltzin *et al.*, 1994). Evidence also showed that brain 5-HT is influenced by the supply of TRP to brain (Stancampiano *et al.*, 1997; Lieben *et al.*, 2004). Bergqvist *et al.*, 1996 reported that single injection of TRP did not alter 5-HT and 5-HIAA levels in rats. Previously in our laboratory it was reported that TRP injected at dose of 50 mg/kg for 4 days caused increase in 5-HT and 5-HIAA levels (Haleem *et al.*, 1998). Other studies reported that TRP rich diet increased TRP/ LNAA ratio (Beulens *et al.*, 2004; Markus *et al.*, 2005) and increased 5-HT metabolism (Feurte *et al.*, 2001; Markus *et al.*, 2002). TRP depletion studies stated that 2 hour after the oral consumption of TRP free diet caused significant reduction in brain TRP and 5-HT levels (Stancampiano *et al.*, 1997a,b; Lieben *et al.*, 2004). However, in the present study single oral administration of TRP at a dose of 100 mg/kg did not increase brain TRP, 5-HT and 5-HIAA levels after 2 hours.

Diet induced increase in TRP availability increases brain serotonergic activity and improves cognitive performance (Schmitt *et al.*, 2000,2005; Booij *et al.*, 2006). The role of serotonin in learning and memory is now well established. The relationship between serotonin and learning and memory has been reported earlier (Khaliq *et al.*, 2006). Increased brain TRP and serotonin levels have been shown to improve cognitive performance (Haider *et al.*, 2006; Haider *et al.*, 2007) whereas decrease in brain serotonin levels has been shown to decrease memory functions (Haider *et al.*, 2005). In the present study lack of any effect on memory functions after single TRP administration may be attributed to the lack of any effect

on 5HT metabolism. Repeated TRP intake in general increases brain serotonin metabolism and increases memory function in rats. These results suggest that this effect of TRP is probably mediated via increased serotonergic neurotransmission. The discrepancy in the results emphasizes that dose and route of administration (i.p. or oral) is important in TRP related experiments.

In light of our previous and present findings we report that increased brain 5-HT metabolism by repeated but not single oral TRP administration is implicated in the enhancement of memory functions in rats.

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