Pak. J. Biochem. Mol. Biol. 2015; 48(3): 74-78

Anxiolytic profile of tryptophan as monitored following repeated administration in male rats

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Abstract: Serotonergic neurotransmission corresponds with the locomotive activity and the elevation in its neurotransmission has an influence on it. Conversely, reduced brain 5-HT undermines the behavioral performance. Serotonin is known to play a central role in physiological responses but also involves regulating mood and behavior. It is the by-product of Tryptophan which is classify as essential amino acid. The present study was therefore destining to evaluate the effects of repeated administration of tryptophan in rat model of depression. Present study revealed that oral administration of tryptophan at the dose of 100 mg/kg for 14 days increased locomotive activity in familiar but decreased in novel environments on repeated administration but not on single administration. Repeated administration of tryptophan were reported to elicit anxiolytic effects in light dark box and elevated plus maze. Results suggest that Anxiolytic effects produced upon repeated administration but not on single administration.

Keywords: Tryptophan (TRP), serotonin, behavioral assessment, locomotive activity, exploratory activity.
Received: June 10, 2015 **Accepted**: August 22, 2015
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INTRODUCTION

In some developed countries, the lifetime prevalence of depression in general population Antidepressants instantly effect on brain functioning by exerting their impact on monoamines, but still pharmacotherapy treatment of depression is consider as time consuming[1], it indicates that along with enhancing monoamine level, some other mechanisms also participates in restoring the normal mood by antidepressants[2-4]. Serotonin (5-hydroxytryptamine, 5-HT), a biogenic monoamine, familiar to modulate numerous functions like anxiety[5], control of mood, food intake and sexual behavior[6].

Tryptophan (TRP) is an essential amino acid, the source of which is diet only. It is the precursor of neurotransmitter serotonin (5-HT). The Synthesis 5-HT in brain rely on the uptake of TRP, which ultimately depends on the plasma ratio of TRP to large neutral amino acids (LNAAs) which compete for the same transport system in the brain[7]. Elevated concentration of TRP increases plasma TRP/LNAAs ratio and triggers the brain serotonin metabolism[8].

Previous studies substantiates that diet with inadequate tryptophan induces anxiogenic and depressive effect on rat behavior[9], while acute depletion elevates the emotional responsiveness of adult rats in stressful condition[10]. Oral administration of tryptophan has been reported to amplify the synthesis and metabolism of 5-HT in different brain regions in rats [11-13], shown to boost cognition[13] and potentiate adaptation to stress[12]. In this regard, tryptophan has probable therapeutic effect in enhancing serotonin concentration and availability of its precursor in the brain, consider as beneficial for the treatment of anxiety and mood disorders. Therefore, the present study was designed to monitor the effect of repeated administration of TRP (100 mg/kg) on different behavioral models of male rats.

MATERIALS AND METHODS

Animals

Locally bred male Albino-Wistar rats (weighing 180-220g) were purchased from The Aga Khan University, Karachi, Pakistan. Animals were housed individually, allowed to acclimatize to their surrounding under 12-hrs light-dark cycle and controlled room temperature (25±2) with free access to cubes of standard rodent diet and water, a week before experimentation. They were also accustomed to various handling procedures to nullify the effects of stress.

Drugs and doses

Tryptophan, purchased from Merck Company was dissolved in distilled water and administrated orally at a dose of 100 mg/kg and control animals were administrated with water by using stainless steel feeding tubes.

Experimental protocol

Twenty four animals were randomly divided into two equal groups (i) water treated and (ii) tryptophan treated. The animals were given orally water or TRP (100 mg/kg) daily for two weeks. Locomotive activities in familiar (activity box) and novel environment (open field), anxiolytic activity in light dark box (time spent) and in an elevated plus maze were monitored 24 hrs after the 1st, 7th and 14th administration of TRP.

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Behavioral assessment

Activity box

To monitor the changes in locomotor activity in familiar environment caused by repeated administration of TRP, animals were introduced in home cage activity box which is consisting of transparent perspex (26 x 26 x 26 cm) bedded with saw dust to provide familiar environment. Testing was done in a quiet room under white light as described by[14, 15], 15 minutes before monitoring the activity animals were placed in the home cage for habituation. Numbers of cage crossings were monitored for 10 minutes.

Open field activity

To assess the effect of TRP on exploratory activity, animals were exposed to the open field apparatus. Animals were individually introduced in open field, which is consisted of a square area (76 x 76 cm) with walls 42 cm high. The floor was divided into 25 equal squares. Procedure was same as described earlier [16, 17] . To determine the activity rats was placed in the center squares of the open field. Numbers of square crossed with all four paws were recorded for 5 minutes.

Light dark box activity

Activity in a light-dark box is used as animal model of anxiety [18]. Tryptophan induced anxiolytic effect were monitored after placing the animals in light dark box which is made up of two equal compartments with size (26x26x26cm), with an access (12x12cm) between the compartments, differed in their sensory properties. One compartment is transparent and exposed to the light while other is black to provide dark area. To perform this test, experimental animals placed in the middle of the light compartment. Number of entries in light compartment with all four paws as described [19] and time spent in the light compartment were monitored for a cut off time of 5 min.

Elevated plus maze test

For the assessment of the effect of repeated administration of TRP on anxiety, EFM apparatus used in the present investigation was specially designed in our laboratory and it consists of four arms in which two were open and two were closed. The arms were of identical length (50 cm) and width (10 cm). Arms were joined by central area of 5 cm^2. The maze was elevated from the floor as a height of 60 cm. To determine the activity a rat was placed in the center of the plus maze and time spent and the entries in the open arm were determined for 5 mints.

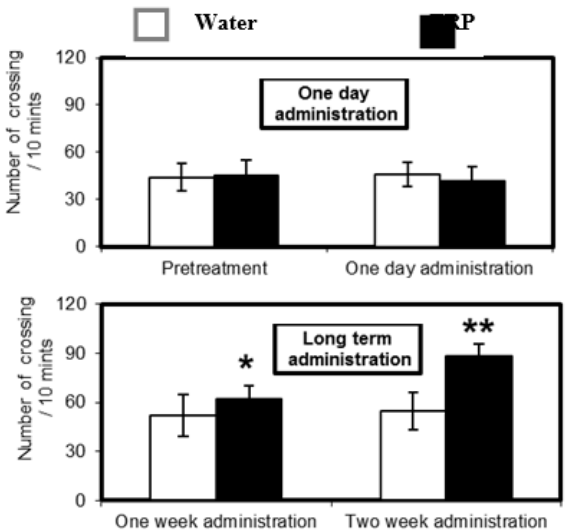
Statistical analysis

Results are represented as mean±SD. SPSS (version 17.0) was used for statistical analysis. Data was analyzed by two-way ANOVA (repeated measures design). Individual comparisons were made by Newman-Keuls test. Values of $p<0.05$ were considered as significant.

RESULTS

Figure 1 shows effects of oral administration of tryptophan on 24 hrs and weekly activity in activity box (cage crossing). Data analyzed by two-way ANOVA (repeated measures design) showed that effects of tryptophan ($F=17.13$; df= 1, 22; $p<0.01$), repeated monitoring ($F=39.885$; df= 3, 22; $p<0.01$) and interaction between the tryptophan administration and repeated monitoring ($F=16.202$; df= 3, 66; $p<0.01$) were significant. Post-hoc analysis by Newman-Keuls test showed no significant change in activity of water treated animals. Activity in tryptophan treated animals was enhanced then water treated animals and values after two weeks treatment were significantly increased in tryptophan treated animals.

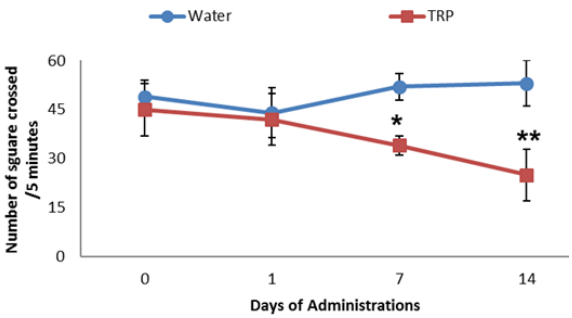
Figure 1: Effects of repeated administrated tryptophan (100 mg/kg) on the activity (cage crossing) of rats in activity box.



Values are means ± SD (n=12) as monitored 24 hrs after the administration. Significant differences by Newman-Keuls test: *$p<0.05$, **$p<0.01$ from water treated controls following two-way ANOVA (repeated measures design).

Figure 2 shows effects of oral administration of tryptophan on 24 hrs and weekly activity in open field (square crossing). Data analyzed by two-way ANOVA (repeated measures design) showed that effects of tryptophan ($F=15.116$; $df= 1, 22$; $p<0.01$) were significant. However, effects of repeated monitoring ($F=0.114$; $df= 3, 22$) and interaction between the tryptophan administration ($F=2.007$; $df= 3, 66$) were not significant. Post-hoc analysis by Newman-Keuls test showed no significant change in activity of water treated animals. Numbers of square crossed in tryptophan treated animals were decreased then water treated animals and values after one and two weeks treatment were significantly smaller in tryptophan treated animals.

Figure 2: Effects of tryptophan (100 mg/kg) administration on activity (square crossing) of rats in open field.

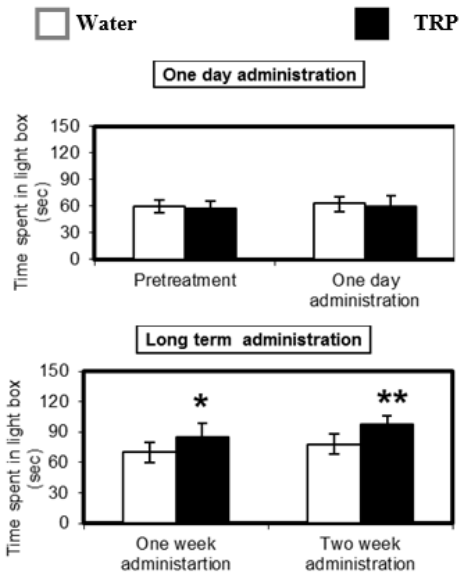


Values are means±SD (n=12) as monitored 24 hrs after the administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from water treated controls following two-way ANOVA (repeated measures design).

Figure 3 shows effects of repeated administration of tryptophan (14 days) on activity in light dark box of rats. Data on time spent in light box as analyzed by two-way ANOVA (repeated measures design) showed that effect of tryptophan ($F=7.076$; $df= 1, 22$; $p<0.05$), repeated monitoring ($F=36.44$; $df= 3, 22$; $p<0.01$) and interaction between the two ($F=7.141$; $df= 3, 66$; $p<0.05$) were significant. Post-hoc analysis by Newman-Keuls test showed that no significant change were found on repeated monitoring in water treated animals. Time spent in light box of tryptophan treated animals was increased then water treated animals and values after one as well as two weeks treatment were significantly higher in tryptophan treated animals.

Figure 4 shows effects of repeated administration of tryptophan (14 days) on activity in elevated plus maze of rats. Data on the time spent in open arm as analyzed by two-way ANOVA (repeated measures design) showed that effects of repeated monitoring ($F=26.061$; $df= 3, 22$; $p<0.01$) and interaction between the two ($F=8.620$; $df= 3, 66$; $p<0.01$) were significant. Whereas, the effects of tryptophan ($F=2.381$; $df= 1, 22$) was not significant. Post-hoc analysis by Newman-Keuls test showed that no significant change in water treated animals. Time spent in open arm was increased in tryptophan then water treated animals and values were significantly higher after two weeks treatment in tryptophan treated animals.

Figure 3: Effects of repeated administrated tryptophan on the activity (time spent) of rats in light dark box.



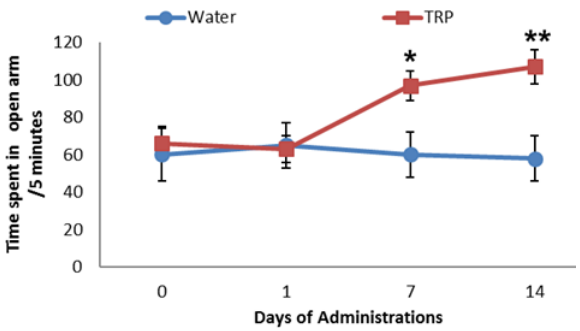
Values are means±SD (n=12) as monitored 24 hrs after the administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from water treated controls; following two-way ANOVA (repeated measures design).

DISCUSSION

It was reported earlier that alteration in brain serotonin levels was also associated with the levels of TRP (its precursor), in the brain[20], as altered supply of TRP to the brain may affect the synthesis of brain 5-HT[21, 22]. In the present study, we investigated the behavioral effects of repeated TRP administration. From the present study, it was demonstrated that TRP induced brain 5-HT metabolism do not applicable to enhance functional serotonergic activity[12]. Results from this study are capable to show that repeated TRP administration at the dose 100 mg/kg enhanced the motor activity in familiar environment whereas it caused hypolocomotion in novel environment while the acute administration did not seen to be effective either increase or decrease in any environment. Anxiolytic behaviors were found on repeated administration of TRP in light dark as well as in

elevated plus maze models. Serotonin (5-HT) centrally regulates physiological responses. Lucki in 1998 have shown that 5-HT also regulates mode and behavior[23].

Figure 4: Effects of repeated administrated tryptophan on the activity (time spent) of rats in elevated plus maze.



Values are means±SD (n=12) as monitored 24 hrs after the administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from water treated controls; following two-way ANOVA (repeated measures design).

Previous study interpreted that serotonin neurotransmission affected by dietary composition. Serotonin is synthesis in the presence of its precursor, tryptophan though it is essential amino acid thereby only available from diet. Consuming the diet with the proportion of carbohydrate and protein can alter the brain 5-HT neurotransmission [24, 25], thereby affecting appetite regulation. Consuming carbohydrates elevate the level of brain TRP due to the fact that diet with carbohydrate profile acquired the insulin-mediated reduction in plasma levels of the large neutral amino acids (LNAA; tyrosine; phenylalanine; valine; leucine; isoleucine) that compete with TRP for uptake into the brain. Dietary protein tended to nourish the bloodstream with massive amount of LNAA. Evidences with valid aggregation from animal and healthy human studies [24-30] shows that restricted diet significantly drops the plasma level of TRP, thereby reduced plasma ratio of TRP to LNAA and eventually diminish the access of TRP in brain. As a consequence, synthesis of 5-HT in brain is reduced, the density of 5-HT transporters down-regulates[31], which initiates the compensatory action of super sensitivity of postsynaptic receptors to reduced 5-HT turnover[32].

The present study shows that activity in familiar environment (Figure 1) is greater in TRP treated rats (repeated administration for two weeks). This increase in activity was observed following repeated but not acute treatment with tryptophan. This might be because of the reason that single administration of TRP could not potentially affect the 5-HT metabolism[33]. Exploratory activities in open field, as monitored after two weeks, were decreased in TRP treated rats (Figure 2). From previous study, it has been reported that low dietary tryptophan seems to have an anxiogenic and depressant effect on rat behavior [9], whereas acute depletion of tryptophan supply in adult rats resulted in increased emotional responsiveness to stressful condition [10]. Anxiolytic effects of TRP were monitored in rats, as assessed by using light dark box as well as elevate plus maze test. In this study, the rats were given TRP at moderate dose showing increased anxiogenic effects following single injection and anxiolytic behavior following repeated administration (Figures 3 and 4). This might be explained in terms of differential effects of TRP on 5-HT metabolism, after single and repeated administration. As alteration in brain 5-HT levels is associated with the changing levels of TRP in the brain [20], single administration of TRP does not affect the formation of 5-HT and 5-HIAA [33]. Whereas, repeated administration of TRP increases 5-HT and 5-HIAA levels[12].

CONCLUSION

Present study reveals that repeated administration of tryptophan produces anxiolytic effects. Findings could be implicated for the treatment of anxiety and/or depression. Since repeated administration of tryptophan is shown to produce anxiolytic effects, TRP should be used as adjuvant therapy initially and later, it could be given alone. Maintenance of anxiety by TRP is advantageous, since it would have less/ no side effects as compared to other drugs currently in use, for the treatment of anxiety. Management of anxiety by TRP is beneficial in a fact that there is less/ no side effect as compared to other conventional drugs which are commonly use, for anxiety

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