Neurochemical and Behavioral Effects of 8-OH-DPAT Following Long Administration of Tryptophan in Rats

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Abstract: Brain tryptophan elevation is the direct response of increased plasma tryptophan. 5–Hydroxytryptamine (5-HT; Serotonin) is synthesized by the hydroxylation of tryptophan. The concentration of 5-HT depends on concentration of its precursor tryptophan. The present study was designed to monitor neurochemical and behavioral effects of a selective 5-HT_{1A} agonist 8-hydroxy-2-di-n-proplyamino tetralin (8-OH-DPAT) at a dose of 0.25 mg/kg following long-term administration of tryptophan in rats. Tryptophan (300 mg/ml/kg) was given orally to rats of test group for a period of two weeks while control rats were given drinking water. Test and control rats were again divided into saline and 8-OH-DPAT injected rats. After 8-OH-DPAT injection, cage crossing, forepaw treading and flat body posture were monitored for 25 minutes. One hour after injection animals were decapitated and plasma & brain samples were collected. Results showed that tryptophan treated animals exhibited higher level of tryptophan in plasma as well as in brain. Injection of 8-OH-DPAT decreased brain tryptophan levels only in tryptophan treated but not in water treated animals. Tryptophan treated animals exhibited higher levels of 5-HT and 5-HIAA. 8-OH-DPAT injection decreased 5-HIAA levels in water treated animals while both 5-HT and 5-HIAA decreased in tryptophan treated animals. Decrease in 5-HIAA levels was greater in tryptophan than in water treated animals. Intensity of 8-OH-DPAT induced serotonin syndrome was comparable in the two groups. Findings are discussed in context of a role of long-term administration of tryptophan on 5-HT_{1A} receptor dependent responses.

INTRODUCTION

A dysfunction of 5-Hydroxytryptamine (5-HT; Serotonin) is the underlying cause for major depressive disorders [1]. Stress and gender differences also determine the vulnerability of a person for depression [2]. It is well known that synthesis of 5-HT in the adult brain is dependent upon the availability of its precursor amino acid tryptophan to the serotonergic neurons [3]. Supply of tryptophan to brain depends upon the concentration of tryptophan present in blood [3]. Injection of tryptophan and drug that affect tryptophan metabolism could change total tryptophan both in plasma and brain [4,5].

Administration of tryptophan stimulates not only the synthesis but also the release of 5-HT [6]. It is generally accepted that reduction in the neuronal function of 5-HT leads to an anxiolytic effect and that increase of 5-HT function result in anxiogenic profile [7,8]. Increasing serotonin functions produces hyperactivity. Serotonin hyperactivity is often described as serotonin syndrome [9-11].

A potential role of 5-HT_{1A} agonists in the enhancement of motor activity was suggested because stimulation of somatodendritic receptors by a selective 5-HT_{1A} agoinst 8-hydroxy-2-di-n-propylaminotetralin (8-OH-DPAT) inhibits 5-HT synthesis to decrease its release from the nerve endings [4,12]. These 8-OH-DPAT induced decrease of 5-HT synthesis therefore gives a measure of presynaptic responsiveness [13,14]. Stimulation of postsynaptic 5-HT_{1A} receptor by 8-OH-DPAT elicits hyperactivity syndrome that is taken as a measure of postsynaptic receptor responsiveness [11,15,16].

Previous studies showed that tryptophan loading increase plasma TRP/LNAA ratio and increases brain tryptophan, 5HT and 5-HIAA levels and therefore increases brain Serotonin activity [16]. The present study was designed to investigate the neurochemical and behavioral effects of 8-OH-DPAT following long term administration of tryptophan.

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats weighing 200- 250 grams were used for the experiment. Animals were caged individually in plastic cages with free access to cubes of standard rodent diet and tap water for one week before starting the experiment.

Drugs

8-OH-DPAT purchased from Research Biochemicals (RBI, USA) was dissolved in saline and injected intraperitoneally at a dose of 0.25 mg/ml/kg. Control animals were injected with saline. L-Tryptophan purchased from MERCK was given daily orally (300 mg/kg/ml).

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Experimental Protocol

24 rats were divided into two groups each containing 12 animals each: (i) water treated and (ii) tryptophan treated rats. Oral tryptophan (300 mg/kg/ml) and water (ml/kg) were given daily for a period of 14 days. On 15th day rats were again divided into saline- and 8-OH DPAT injected rats, resulting in four groups each containing six animals: (i) Water-saline (ii) Water-8-OH-DPAT (iii) Tryptophan-Saline (iv) Tryptophan-8-OH-DPAT injected rats. Rats were then injected with saline (1.0 ml/kg) or 8-OH-DPAT (0.25mg/ml/kg) respectively. 5minutes after injections, 8-OH-DPAT-elicited 5-HT syndrome was monitored (cage crossing, forepaw treading and flat body posture) for 25minutes. 1 hr post injection, rats were decapitated and both plasma and brain samples were collected immediately and stored at -70^{o}C until analysis.

8-OH-DPAT Elicited 5-HT Syndrome

Animals were placed individually in skinner's box (transparent perspex cages with area of 26x26x26 cm and sawdust covered floor) 15 minutes before injecting 8-OH-DPAT. Rats were injected in a balanced design. Flat body posture, forepaw treading and locomotion elicited by the drug were scored 5 min post injection for 25 minutes, as described earlier [15].

Statistical Analysis

Results are presented as ± SD. Data was analyzed by two-tailed student's t-test and two-way ANOVA. Post-hoc comparisons were made by Newman-Keuls test. Values of $p<0.05$ were considered significant.

RESULTS

Fig. (**1**) shows 8-OH-DPAT-induced hyperactivity and fore paw treading in water+saline, water+8-OH-DPAT, tryptophan+saline and tryptophan+8-OH-DPAT treated animals. Data analyzed by student's t-test showed a non-significant effect 8-OH-DPAT following long term administration of tryptophan on fore paw treading and cage crossing as monitored in skinner's box. Whereas, significant effects of -OH-DPAT following long term administration of tryptophan were observed on flat body posture.

Fig. (**2**) shows plasma and brain tryptophan levels in water+saline, water+8-OH-DPAT, tryptophan+saline and tryptophan+8-OH-DPAT treated animals. Data on plasma tryptophan levels (Fig. **2a**) as analyzed by two-way ANOVA showed that effect of 8-OH-DPAT on plasma tryptophan was not significant (df= 1,20; F= 0.057). While effects of tryptophan administration (df= 1,20; F= 36.3; $p<0.01$) and interaction between two factors were significant (df= 1,20; F= 6.8, $p<0.05$). Post-hoc analysis by Newman-keuls test showed increased ($p<0.01$) plasma tryptophan in tryptophan-saline treated animals as compared to water-saline injected controls. Levels of plasma tryptophan were also increased ($p<0.05$) in tryptophan-saline treated animals as compared to water-saline injected controls.

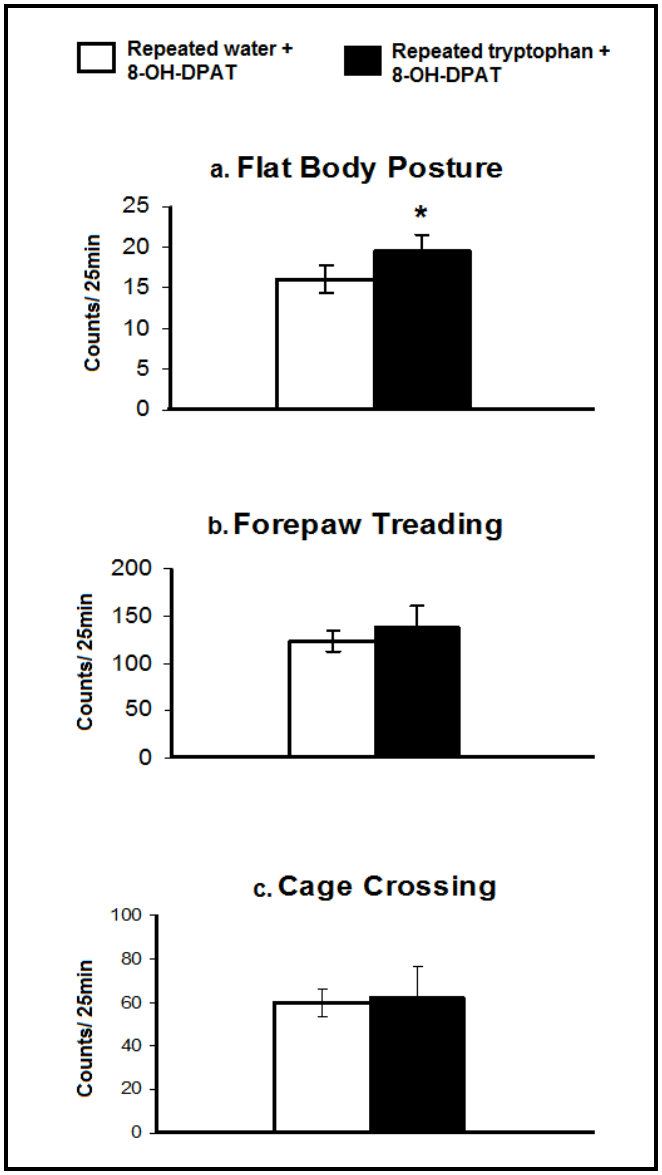

Fig. (1). Effects of 8-OH-DPAT (0.25 mg/kg/ml) following two weeks of administration of tryptophan on flat body posture, forepaw treading & cage crossing in skinner's box. Values are means ± SD (n = 6). *$p<0.05$ following two-tailed t-test were non-significant.

Data on brain tryptophan levels (Fig. **2b**) as analyzed by two-way ANOVA showed a significant effect of tryptophan (df= 1,20; F= 112.9; $p<0.01$), 8-OH-DPAT (df= 1,20; F=5.62; $p<0.05$) and interaction between the two (df= 1,20; F= 24.7; $p<0.01$) on brain tryptophan levels of rats. Post-hoc analysis by Newman-keuls showed that tryptophan administration significantly increased ($p<0.01$) brain tryptophan in tryptophan-saline treated animals as compared

to water-saline treated animals. 8-OH-DPAT decreased ($p<0.05$) brain tryptophan levels in tryptophan-8-OH-DPAT treated animals as compared to tryptophan-saline controls. Whereas brain tryptophan levels in tryptophan-8-OH-DPAT treated animals were greater ($p<0.01$) than water-8-OH-DPAT injected animals.

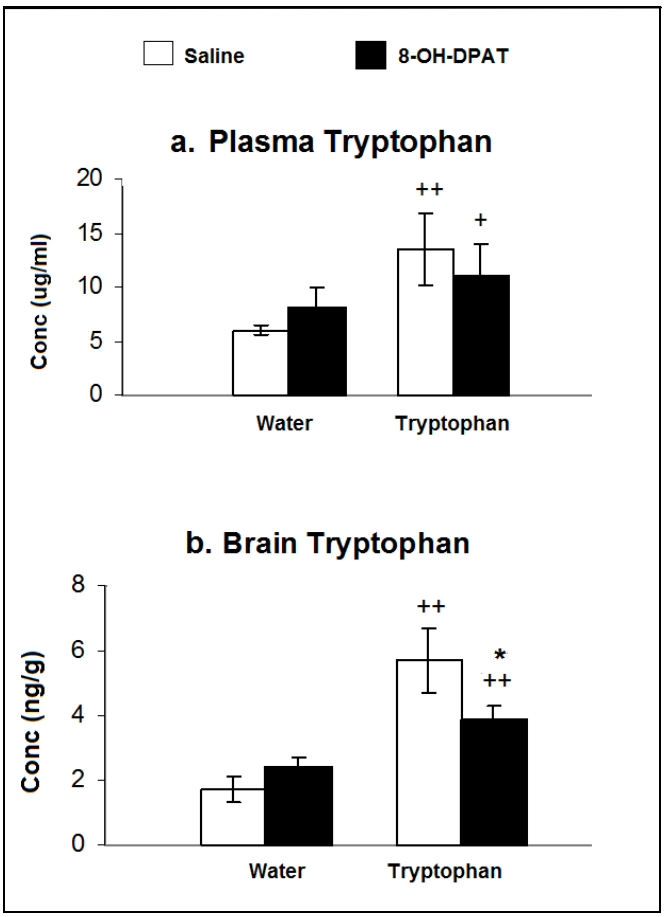

Fig. (2). Effects of 8-OH-DPAT (0.25 mg/kg/ml) following two weeks of administration of tryptophan on plasma & brain tryptophan in rats. Values are means ± SD (n = 6). Significant differences by Newman- Keuls test: +p<0.05, ++ p<0.01 from respective water treated control and *p<0.01 from respective tryptophan treated rats following two-way ANOVA.

Fig. (**3**) shows brain 5-HT and 5-HIAA levels in water+saline, water+8-OH-DPAT, tryptophan+saline and tryptophan+8-OH-DPAT treated animals. Data on brain 5-HT levels (Fig. **3a**) as analyzed by two-way ANOVA showed that effects of 8-OH-DPAT on 5-HT level were not significant (df= 1,20; F= 0.003). However, effects of tryptophan (df= 1,20; F= 25.4; $p<0.01$) and interaction between the two factors were significant (df= 1,20; F = 9.8, $p<0.05$). Post-hoc analysis by Newman-Keuls test showed that 8-OH-DPAT increased ($p<0.01$) 5-HT levels in tryptophan+saline and tryptophan+8-OH-DPAT treated animals as compared to respective water-saline and water-8-OH-DPAT treated controls. These increased levels of 5-HT in tryptophan+8-OH-DPAT treated animals were attenuated ($p<0.05$) by 8-OH-DPAT as compared to tryptophan+saline treated controls.

Data on brain 5-HIAA levels (Fig. **3b**) as analyzed by two-way ANOVA showed significant effect of 8-OH-DPAT (df= 1,20; F=12.6, $p<0.01$) on brain 5HIAA level of rat. Effects of tryptophan (df= 1,20; F=1.9) and interaction between the two (df= 1,20; F=2.6) were found to be non-significant. Post hoc analysis by Newman Keuls test showed that brain 5HIAA levels were decreased in by 8-OH-DPAT in both water+8-OH-DPAT and tryptophan+8-OH-DPAT treated animals as compared to their respective water+saline and tryptophan+ saline treated controls. Tryptophan administration significantly ($p<0.05$) increased 5HIAA level in saline injected as compared to water+saline injected controls.

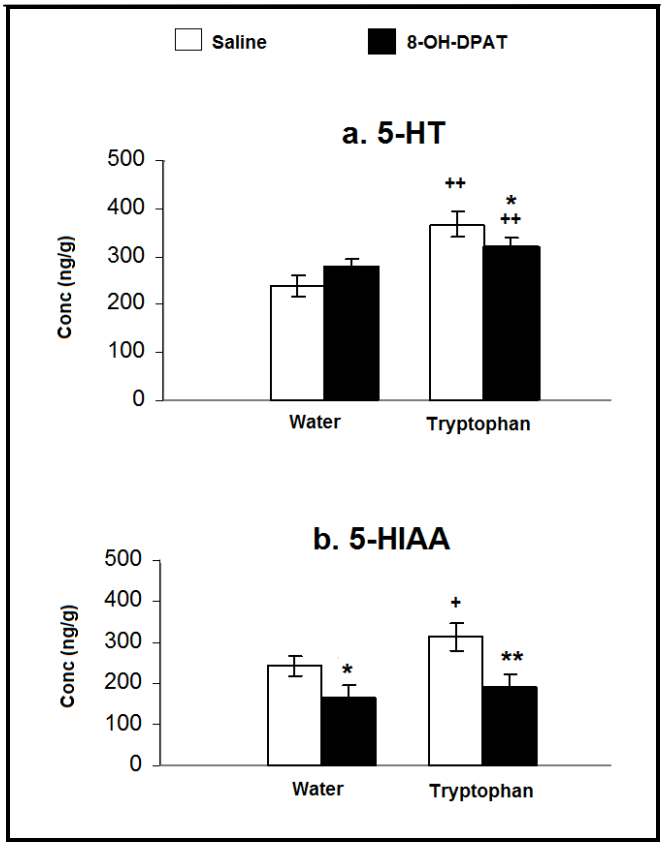

Fig. (3). Effects of 8-OH-DPAT (0.25 mg/kg/ml) following two weeks administration of tryptophan on Brain 5-HT & 5-HIAA levels in rat brain samples. Values are means ± S.D. (n=6). Significant differences by Newman- Keuls test: ++p<0.01, +p<0.05 from respective water treated control and **p<0.01, *p<0.05 from respective tryptophan treated rats following two-way ANOVA.

DISCUSSION

Administration of tryptophan increases both brain tryptophan and brain 5-HT levels. The concentration of 5-HT in the brain totally depends upon the concentration of

tryptophan in the plasma [4]. Present study was designed to investigate the effect of long administration of tryptophan (for two weeks) orally at a dose of 300 mg/kg/ml following the injection of 8-OH-DPAT in rats. Increasing Serotonin functions produces hyperactivity [10].

Serotonin hyperactivity is often described as Serotonin syndrome, which involves certain Stereotype behaviors as gnawing head twitch, head weaving, forepaw treading, hind limb abduction and Straub tail [9]. Reduction in the neuronal functions of 5-HT leads to an anxiolytic effect and increase of 5-HT function result in anxiogenic effect [18]. 5-HT_{1A} receptors located on 5-HT nerve cell bodies and / or dendrites in the raphe nuclei, function as auto receptors. Thus stimulation of 5-HT_{1A} receptors in the raphe nuclei inhibits the firing of 5-HT neuron and causes a decrease in the release of 5-HT in terminal fields [19-21] and increased 5-HT metabolism [17]. Drugs effects in the enzymes, which are involved in the synthesis or degradation of neurotransmitter amine.

Drugs can inhibit or activate the enzymatic activity, which is involved in the synthesis or degradation of Serotonin. As a result of which synthesis or degradation of Serotonin inhibit or accelerate. These drugs alter the serotonergic functions and alter release or high affinity reuptake from the synaptic cleft. Different Serotonin against as 8-OH-DPAT which is selectively 5-HT_{1A} against its administration, in rats has been shown to decrease the synthesis rate and turnover of 5-HT in the brain of rat [12,19]. This decrease in Serotonin turnover by against may result from a greater stimulation of pre-synaptic auto-receptors on Serotonin neurons or from the stimulation of postsynaptic receptors leading to transneural feedback influences on the serotonin neurons [12,22]. In the previous studies it has been shown that administration of tryptophan increased plasma and brain tryptophan concentration. Administration of 100 mg/kg tryptophan produced a rise in plasma and brain tryptophan and increase the brain 5-HT synthesis [23].

Previously, it was also reported [16] that administration of 100 mg/kg tryptophan orally given to rats for 6 weeks increased plasma tryptophan, brain tryptophan, 5-HT and 5-H1AA levels. In the present study when the tryptophan was orally given for two weeks at a dose of 300 mg/kg, it was observed that plasma and brain tryptophan was increased significantly, which in turns enhances the synthesis of brain 5-HT and 5-HIAA consistent with the previous studies. In the present study it was also observed that tryptophan treated animals exhibit higher levels of plasma tryptophan as well as brain tryptophan. Injections of 8-OH-DPAT decreased brain tryptophan level of rats only in tryptophan treated but not in water treated animals. 5-HT and 5-HIAA levels are higher in tryptophan treated animals. Decreases of 5-H1AA were greater in tryptophan than in water treated animals.

Thus, present results strengthen the previously reported suggestions that long-term administration of tryptophan increases the synthesis of plasma tryptophan, brain tryptophan, brain 5-HT and its metabolite 5-H1AA and changes the physiological functions to 5-HT.

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