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Behavioral consequences of repeated fluoxetine administration in tryptophan pretreated albino wistar rats

*Hira Awan, Sana Qadeer and Muhammad Farhan**
Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry
University of Karachi, Karachi, Pakistan

Abstract: Serotonin or 5-hydroxytrypamine (5-HT) is the well-known neurotransmitter extensively distributed in the CNS. Mainly 5-HT and its related drugs are used in psychiatry and neurology. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants. Fluoxetine, an SSRI blocks serotonin reuptake in to presynaptic neurons. Hence, working as an antidepressant. Tryptophan, an essential amino acid is precursor of serotonin i.e. 5-HT. Both, fluoxetine and tryptophan interfere with serotonergic neurotransmission. So, the objective of present study was to observe the effects of repeated fluoxetine administration on rat behaviors that pretreated with tryptophan for a week. Fluoxetine administered animals that were pretreated with tryptophan shown more locomotors activity in activity box and open field than rats solely treated with tryptophan. Anxiolytic behavior of fluoxetine was observed in tryptophan pretreated animals were greater. The results we got may prove to be beneficial for the treatment of multiple mood disorders including depression and anxiety in a way that tryptophan can be given along with fluoxetine for better outcomes.

Keywords: 5-Hydroxytryptoamine, selective serotonin reuptake inhibitors, locomotive activity, anxiolytic behavior, fluoxetine.
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***Author for Correspondence**: farhankamali@uok.edu.pk

INTRODUCTION

Serotonin (5-Hydroxytryptamine 5-HT) is known to play a neurotransmitter role in the adult brain, modulating emotion, learning, cognition, sleep and stress responses[1.] During neurodevelopment, serotonin is implicated in several processes, such as cell division, migration, differentiation, growth, cone elongation, myelination, dendritic pruning and synaptogenesis[2]. Serotonin modulates the development of its own and other neuronal systems, including the HPA axis[3, 4]. Exposure to SSRIs during development results in an acute increase in serotoninergic tone[5]. However, perinatal SSRI exposed animals have shown reductions in serotonin levels during adulthood, through developmental activation of inhibitory autoreceptors (e.g. 5-HT-1A)[5,6]. The discovery of selective serotonin reuptake inhibitors (SSRIs) has emerged as a major therapeutic advance in psychiatry and marked a milestone in psychopharmacology. The first of the SSRIs, fluoxetine (FLX), was introduced in the United States in 1988 and has launched a new era in psychotropic drug therapy[7]. SSRIs, including fluoxetine, sertraline, paroxetine, citalopram and fluvoxamine, are widely prescribed for a range of behavioral and psychiatric problems.

In combination with tricyclic antidepressants, SSRIs offer a potent therapy for fibromyalgia[8]. A side effect of SSRIs coincidentally provides therapy for premature ejaculation[8]. Selective serotonin reuptake inhibitors are widely used because of their safety, tolerability, and demonstrated efficacy[9]. Adverse effects as well as therapeutic effects of SSRIs appear to exhibit topography with neuro-anatomic site and pathway specific actions for each physiological function and involving different receptor subtypes[10]. With the clarification of multiple discrete projection pathways for serotonin in the central nervous system, research is attempting to map topography of serotonin physiology by elucidating which function (s) any given pathway controls[11-13].

Tryptophan (TRP), an essential amino acid and precursor of 5-HT[14]. Oral TRP administration increases plasma TRP and Large neutral amino acids ratio and enhances brain serotonin activity[15]. It has been proved that TRP depletion produces an anxiogenic and depressant effect on rat behavior[16]. On the other hand, acute depletion in adult rats seems to increase the emotional responsiveness to stressful situations[17]. Along with other physiological functions 5-HT also enhances cognitive functions[18]. Khaliq and colleagues reported that tryptophan at the doses of 50 and 100 mg/kg increases plasma TRP levels and brain 5-HT metabolism in rats and also improves there cognitive performance[19].

The present study was designed to study the behavioral effects of oral fluoxetine administration on rats pre-treated with tryptophan at dose 100 mg/kg for 7 days. As, both drugs interfere with serotonin levels in synapse.

MATERIALS AND METHODS

Animals

Total 24 male Albino wistar rats weighing 120-180gm were purchased from The Dow University of Health and Sciences, Karachi, Pakistan and housed in individual cages and allowed to acclimatize to their surrounding with temperature (25±2ºC) for one week. Animals had free access to a normal standard diet during acclimatizing time before starting experiment.

Drugs and doses

All chemicals and drug used during the study were purchased from the Sigma Chemical Company (USA) and Merck Company. Tryptophan and Fluoxetine was dissolved in distilled water and administrated orally at their respective doses 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 animals. The animals were given orally water or TRP (100 mg/kg) with the help of small feeding tube made up of stainless steel that is attached to a 1 ml syringe daily for one week. After one week TRP treatment, animals were divided into (i) Water- Water, (ii) Water-FLX, (iii) TRP-Water and (iv)TRP-FLX. Animals were administrated accordingly with Fluoxetine (10mg/kg) and water daily for one week.

Behavioral activity in activity box and light-dark transition box were monitored on next day of the 1st, 3rd, 5th and 7th administration of fluoxetine. Exploratory activity in open field was monitored on next day of 1st and 7th administration of fluoxetine.

Behavioral assessment

Activity box

The assessment of locomotive activity in a familiar environment was done by activity box test. The apparatus used in this study was a square perspex activity cage (26x26x26 cm) with a saw dust covered floor. Testing was done in a quiet room. Before monitoring the activity animal was placed in it for 15 minutes for the habituation. Number of crossing across the cage was monitored for 10 minutes.

Open Field

The determination of exploratory locomotive activity in a novel environment as it may be altered by respective treatments was done by open field activity test. The test consists of measuring the activity of rats in an open novel space, from which escape is prevented by a surrounding wall. The open filed apparatus used in this present investigation consisted of a square area 76x76 cm with opaque walls 42cm high. The floor of apparatus was divided by lines into 25 equal squares**.** To determine the activity a rat was placed in the center squarer of the open field. The latency to move was monitored in seconds and the exploratory activity (number of square crossed with all four paws) were scored for 5 minutes.

Light dark transition box

Light-dark box transition is used as measure of anxiety[20]. The test was conducted in a locally made two compartment box. The compartments of equal size (26 x 26 x 26 cm), with an access (12x12cm) between the compartments, differed in their sensory properties**.** Walls of one compartment were light (transparent) and other dark (black). A rat placed in this box is expected to pass more time in the light compartment. To determine the activity a rat was introduce in the middle of the light compartment of the box. Entries and time spent in the light compartment were monitored for a cut off time of 5 minutes entry into a compartment of the box is defined as the placement of all four paws in the compartment of the activity box. Increased time spent in light compartment is used as an indicator of reduced anxiety states.

Statistical analysis

Results are presented as mean±SD. Data of the fluoxetine administrations on the behavioral models of tryptophan pre-treated animals were analyzed by three–way ANOVA (repeated measures design). Software used for the analysis was SPSS (version 17). Individual comparisons were made by Newman-Keels test. Values of $p<0.05$ were considered as significant.

RESULTS

Figure 1 shows effects of repeated fluoxetine administration at dose of 10mg/kg on activity (number of cage crossed) in activity box of rats pre-treated with tryptophan (100 mg/kg) as monitored on next day of 1st, 3rd, 5th and 7th administration. Analysis of the data by three-way ANOVA (repeated measures design) showed that effect of tryptophan ($F=47.021$; $df=1, 20$; $P<0.01$), fluoxetine ($F=135.956$; $df=1, 20$; $P<0.01$), repeated monitoring ($F=19.907$; $df= 3, 20$; $p<0.05$) and interaction among repeated monitoring, tryptophan and fluoxetine ($F=11.252$; $df=3, 20$; $P<0.05$) were significant. Post-hoc analysis by Newman-Keuls test showed that fluoxetine increased activity in tryptophan as well as water treated animals. Significant ($p<0.01$) increased in activity was found after 5th and 7th administration in water and after 3rd, 5th and 7th administration in tryptophan treated animals. Fluoxetine administration increased number of cage counts in water (5th and 7th) as well as in TRP (3rd, 5th and 7th) treated animal significantly ($p<0.01$) as compared to similarly treated animals after 1st administration. In tryptophan treated animals, fluoxetine increased activity significantly ($p<0.01$) after 3rd, 5th and 7th administration as compared to similarly administrated water treated animals of same day.

Figure 2 shows effects of repeated administration of fluoxetine at dose 10 mg/kg on number of squares crossed in open field of rats pre-treated with tryptophan (100 mg/kg) as monitored on next day of 1st and 7th administration. Analysis of the data by three-way ANOVA (repeated measures design)

showed significant effect of tryptophan (F=134.281; df=1, 20; P<0.01) and interaction between the days, tryptophan and fluoxetine (F=8.856; df= 1, 20; p<0.05). However, the effects of fluoxetine (F= 3.092; df=1, 20) and repeated monitoring (F= 0.624; df=1, 20) were found non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of fluoxetine decreased number of square crossed in water as well as TRP treated animal. Significant decreased in activity was after 1st (p<0.05) and 7th (p<0.01) administration in TRP treated animals. Activity of TRP treated animals was decreased after repeated administration of fluoxetine significantly (p<0.01) as compared to similarly treated fluoxetine administrated animals of single administration. After a week administration, activity in open field was decreased in TRP treated animals (p<0.01) as compared to similarly administrated water treated animals.

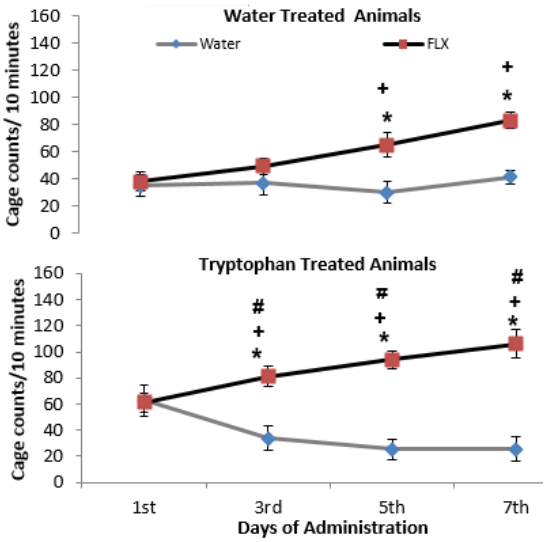

Figure 1: Effects of fluoxetine administration on activity in activity box of rats pretreated with tryptophan. Values are means±SD (n=6) as monitored on next day of 1st, 3rd, 5th and 7th administration. Significant differences by Newman-Keuls test: *p<0.01 from respective water administrated controls; +p<0.01 from similarly administrated water or TRP treated animals of 1st day administration; #p<0.01 from same day water treated fluoxetine or water administrated animals following three-way ANOVA (repeated measures design).

Figure 3 shows effects of repeated fluoxetine administration at dose 10 mg/kg on light dark box activity (time spent in light box) in rats previously treated with tryptophan (100 mg/kg) as monitored on next day of 1st, 3rd, 5th and 7th administration. Analysis of the data by three-way ANOVA (repeated measures design) showed significant effects of Tryptophan (F=98.367; df= 1, 20; p<0.01), fluoxetine (F=39.212; df= 1, 20; p<0.01) and the interaction among tryptophan, fluoxetine and repeated monitoring (F=29.238; df= 3, 20; p<0.01). However, the effects of days (F=2.125; df= 3, 20) was non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of fluoxetine increased time spent in light dark box of both water as well as TRP treated animals. Significant increase in activity was seen after 7th (p<0.05) administration in water treated as well as 5th and 7th administration (p<0.01) in TRP treated animals as compared to respectively water administrated controls.

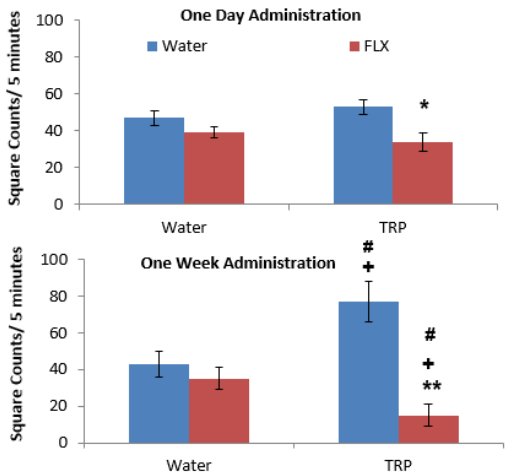

Figure 2: Effects of fluoxetine administration on activity in open field of rats pretreated with tryptophan. Values are means±SD (n=6) as monitored on next day of 1st and 7th administration. Significant differences by Newman-Keuls test: *p<0.01 from respective water administrated controls; +p<0.01 from similarly administrated water or TRP treated animals of same day; #p<0.01 from similarly administrated water or TRP treated animals of 1st day administration following three-way ANOVA (repeated measures design).

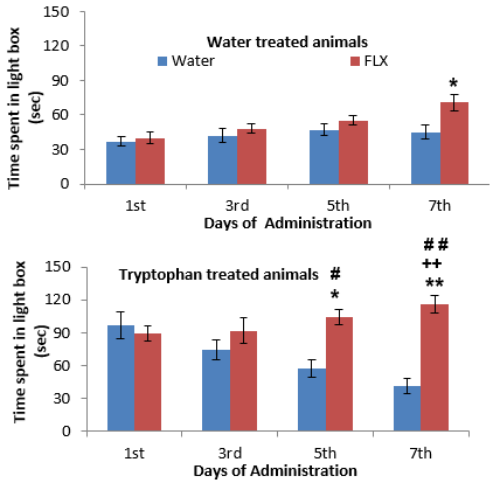

Figure 3: Effects of Fluoxetine on activity in light dark activity box of rats pretreated with tryptophan. Values are means ± SD (n=6) as monitored on next day of 1st, 3rd, 5th and 7th administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective water administrated controls; p<0.05, ++p<0.01 from similarly administrated water or TRP treated animals of 1st day administration; #p<0.05, ##p<0.01 from same day water treated fluoxetine or water administrated animals following three-way ANOVA (repeated measures design).

In TRP treated animals, fluoxetine increased activity after 7th administration ($p<0.01$) as compared to respectively similar administrated animals from 1st day. Fluoxetine administration increases time spent in light box after 5th ($p<0.05$) as well as 7th ($p<0.01$) administration of TRP treated animals as compared to similarly administrated animals of water treated group of same day.

DISCUSSION

The aim of present study was to investigate the effects of fluoxetine, a commonly used antidepressant of SSRI class, at dose of 10 mg/kg/day on rats pretreated with tryptophan repeatedly. To evaluate behavioral effect of fluoxetine on rats different tests were used like light-dark box, open field and activity box. Serotonergic mechanisms play an important role in the modulation of locomotors activity at a number of levels in the neuroaxis including the spinal cord, the basal ganglia, limbic structures, and in the frontal cortex[21-23]. Fluoxetine treated animals shown increased locomotive activity in familiar environment upon repeated administration but animals pre-treated with TRP were more active. Water treated animals shown significant declining activity in animals pre-treated with TRP. Results from present study show that TRP treated animals shown decreased activity in novel environment on acute and sub-chronic FLX administration than water treated animals. FLX increased activity in animals previously treated with water. Repeated and acute administration of SSRIs are known to yield finite beneficial effects or even adverse effects on anxiety and depression[24, 25]. However, chronic SSRIs treatments are effective in depressed or anxious patients[26, 27] as well as in highly emotional animal models[25, 28]. Fluoxetine is devoid of affinity for serotonin receptors[29], but it acts as an indirect agonist, stimulating multiple 5-HT receptors. Because serotonergic neurotransmission is based on multiple 5-HT receptors types and subtypes, 5-HT-1A-1F, 5-HT-2A-2C and 5-HT-3-7[30-33]. The study of the specific blockade of 5-HT receptors could be useful to explain the mechanisms of action of this monoamine on learning and memory. Anxiolytic effects if fluoxetine were monitored in light dark transition. This study indicated that FLX treated rats shown anxiolytic behavior with successive number of doses but tryptophan treated animals shown more significant effects than water treated. On the other hand, water administered animals showed anxiogenic behavior in TRP treated animals It has been reported from present study that, fluoxetine does not produce desired effect on acute administration so it should be administered repeatedly. Results we got may be beneficial for the treatment of multiple mood disorders including depression and anxiety in a way that tryptophan can be given along with fluoxetine for better outcomes. Present study will help to understand the interaction between serotonergic transmissions and depressive situation.

CONCLUSION

The present study conclude that repeated tryptophan administration for a week and then treatment of tryptophan treated rats with fluoxetine for one more week results in increase of locomotor activity. Fluoxetine administration at the dose of 10 mg/kg/day produced anxiolytic effect on rats and these effects were more notable in tryptophan (100 mg/kg) treated animals.

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