REPORT

Attenuation of apomorphine induced behavioral sensitization in rats pre-treated with tryptophan

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Abstract: Psychostimulants substances, some of which are abused by humans, are generally believed to produce sensitization effects when they are repeatedly administered to animals. Apomorphine, a non-narcotic derivative of morphine, having agonistic property for dopamine in order to produce psycho stimulant-like effects. Meanwhile, chronic administration leads to behavioral sensitization. Therefore, present study destine to produce desensitization in animals by the repeated administration of tryptophan (100 mg/kg), thereafter treated with apomorphine (1.0 mg/kg) to observe the intensity of sensitization in rats pre-treated with tryptophan. Apomorphine on acute administration known to increase motor activity whereas repeated treatment of apomorphine initiates the sensitization of motor behavior. It is expected that the intensity of apomorphine induced sensitization would be affected in tryptophan-treated rats. Present study provide the clear-cut evidence that chronic treatment of apomorphine arouses the motor behavior of animals in both novel and anxiolytic model over the saline treated animals, whereas hypo locomotive behavior was seen in animals pre-treated with tryptophan, provides the evidence that preliminary treatment of tryptophan perturbs the apomorphine induced sensitization in animals. The discoveries present an inventive methodology for amplifying the remedial utilization of apomorphine and traditional psychostimulants.

Keywords: Sensitization, Desensitization, Apomorphine, Tryptophan, Locomotor activity.

INTRODUCTION

Psychostimulant are known to yield sensitization on chronic use due to the fact that chronic use of psychostimulants drugs causes the receptors up regulated, this phenomenon is known as sensitization (Keller S *et al.,* 2002**)**. Apomorphine is a potent D_1/D_2 receptor agonist (Wang *et al.,* 2007). Repeated administration of dopamine agonist either direct, on pre synaptic receptor, or indirect, on terminal side accelerates the behavioral effects of drugs and induces sensitization (Kalivas and Stewart, 1991; Mattingly *et al.,* 1977). Repeatedly administrated apomorphine induced behavioral sensitization, which is monitored by a progressive optimization in locomotor behavior (Mattingly *et al.,* 1977) and instantly after the treatment (Hasnat and Haleem, 2005). Kalivas and Stewart (1991) spotlighted on a fact, which is common in all psychostimulants with distinct mechanism of action that the onset of sensitization caused by diminished somatodendritic dopamine (DA) release. Apomorphine at different doses (0.5-5.0 mg/kg) produces stereotyped responses (sniffing, licking and gnawing) in rats and mice and a concomitant period of hyper-locomotive behavior expressed by animals in different situations as climbing and turning behavior (Moller *et al.,* 1987a; Mattingly and Gotsick, 1989; Mattingly *et al.,* 1997; Tirelli and Heidbreder, 1999a; Battisti *et al.,* 2000).

Like amphetamine and cocaine, apomorphine also having potential to produce behavioral stimulation by enhancing the action of DA receptor at certain doses. Apomorphine induced behavioral sensitization is explicitly indexed by the stimulation of dopamine D_1 receptors (Mattingly *et al.,* 1991) and modification of DA synthesis (Rowlett *et al.,* 1991). Behavioral sensitization is associated to sustain enhanced responsiveness extracellular DA level in the neurons that innervate the nucleus accumbens after disrupted administration of psycho-stimulant for at least one week (Kalivas *et al.,* 1991; Heidebreder *et al.,* 1996).

Tryptophan (TRP) is well known large neutral amino acid, which is essential, as it cannot be synthesized inside the body thus, require by diet. The synthesis of serotonin in the brain is based on the uptake of tryptophan, precursor of serotonin. The plasma ratio of tryptophan to large neutral amino acids (LNAA) deserve to attention which is responsible to the uptake of TRP by the competition of transport system. Increased plasma ratio of TRP/LNNA characterized by increased activity of

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serotonin in the brain (Markus *et al.,* 2002). Previous study demonstrates the anxiogenic as well as depressive effects of low dietary TRP in the rat's models (Zhang *et al.,* 2006) while stressful response is experienced by the acute depletion of TRP (Uchida *et al.,* 2005). TRP orally, causes different brain regions to boost the synthesis and metabolism of serotonin (Haleem *et al.,* 1998; Haider *et al.,* 2006, Khaliq *et al.,* 2006) and restore cognition (Khaliq *et al.,* 2006). Dopaminergic agent apomorphine, selective towards the D_1 and slightly greater towards D_2 receptors (Wang *et al.,* 2007) promotes sensitization on chronic administration (1.0 mg/kg) as analyzed either in familiar or novel environment (Haleem *et al.,* 2005; Ikram *et al.,* 2011). Treatment of tryptophan for two week reported to evaluate adaptive response to stress (Haleem *et al.,* 1998) indicates desensitization of somatodendritic 5-HT-1A receptors (Haleem, 1999), conversely, chronic treatment of apomorphine leads to sensitize the somatodendritic 5-HT-1A receptors (Haleem *et al.,* 2005; Ikram and Haleem, 2011). It predicts that preliminary treatment of tryptophan can help to prohibit apomorphine induced sensitization. The present study was therefore, designed to test the hypothesis that administration of tryptophan could attenuate the apomorphine induced sensitization in rats.

MATERIALS AND METHODS

Animals

Albino-Wistar rats (weighing 180-220 grams) provided by the Aga Khan Medical University, Karachi, Pakistan, were housed in individual cages under 12-h light/dark cycle and maintained room temperature (25±2ºC) with free access of water and standard rodent diet, for a period of three days before experimentation.

Drugs and doses

Apomorphine-HCl (Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intraperitoneally at dose of 1.0 mg/kg (Tabaei *et al.,* 2002) to the respective group animals. Drug was freshly prepared before starting the experiment. Saline (0.9% NaCl solution; 1 ml/kg) was injected to control animals.

Experimental protocol

Twenty four animals were randomly divided into two equal groups (i) water treated and (ii) tryptophan treated. The animals were orally administered water or TRP at dose 100 mg/kg (Khaliq *et al.,* 2007) by small feeding tube made up of stainless steel that is attached to a 1 ml syringe daily for two weeks. After two weeks TRP treatment, animals were equally divided into (i) Water-Saline, (ii) Water-Apo, (iii) TRP-Saline and (iv) TRP-Apo. Animals were injected accordingly with apomorphine (1.0 mg/kg) and saline daily for next 14 days. Food intake and growth rate was monitored on next day of every apomorphine injection. Activity in activity box (familiar environment) and open field (novel environment) was monitored after 24 hrs of 1st, 7th and 14th injection of apomorphine. Anxiolytic behavior of the treatment was determined in light-dark transition test and elevated plus maze test, as monitored on next day of the 1st, 7th and 14th injection of apomorphine.

Behavioral assessment

Activity box

The assessment of locomotor activity was done in home cage (activity box). Apparatus made up of transparent perspex (26 x 26 x 26 cm) and floor is covered by saw dust. 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

The exploratory activity was monitored in a novel environment by using open field apparatus to measure anxiety-like behavior and locomotion. Apparatus consisted of a square area (76 x 76 cm) with walls 42 cm high. The floor was consisting of 25 equal squares. Procedure was same as described earlier (Ikram and Haleem, 2011; Ikram *et al.,* 2007). For the determination of effects of treatment the animal was placed in the center square of the open field and the numbers of square crossed with all four paws were counted. Cut off time was 5 minutes.

Light dark box activity

Activity in a light-dark box is used as animal model of anxiety (Shimada *et al.,* 1995). The apparatus consist of two square shape compartments. The compartment of equal size (26 x 26 x 26 cm), with an access (12 x12 cm) between the compartments, one compartment was light and other was dark. To determine the activity, rat was placed in the middle of the light compartment. Number of entries and time spent in the light compartment of the light dark box were monitored. Activities were monitored for a period 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 (Bourin M. Hascoet, 2003).

Elevated plus maze test

The elevated plus maze is used for the assessment of anxiety. The EPM apparatus used in the present investigation was specially designed in our laboratory and it consists of four arms. Two arms 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 height of maze was 60cm from the floor. Animals were placed in the center point of the plus maze to determine the activity. Number of entries and time spent in open arm was recorded. Cut off time was 5 minutes.

STATISTICAL ANALYSIS

Results are presented as means ± SD. Data on the effects of apomorphine on the behavior in water and tryptophan 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-Keuls test. Values of $p<0.05$ were considered as significant.

RESULTS

Fig. 1 show effects of repeated administration of apomorphine at dose 1.0 mg/kg on numbers of cage crossing in activity box of rats pre-treated with tryptophan. Data analyzed by three-way ANOVA (repeated measures design) showed that effects of repeated monitoring ($F=18.784$; df=2, 42; $p<0.01$), apomorphine ($F=421.52$; df=1, 21; $p<0.01$) and interaction between all the factors ($F=17.81$; df= 2, 42; $p<0.01$) were significant. Whereas, the effects of tryptophan ($F=1.31$; df= 1, 21) was not significant. Post-hoc analysis by Newman-Keuls test showed that apomorphine increased locomotor activity as number of cage crossing increased after one day administration and values were significantly greater in water treated animals. Apomorphine-induced activity was greater after one week and two weeks administration than single administration

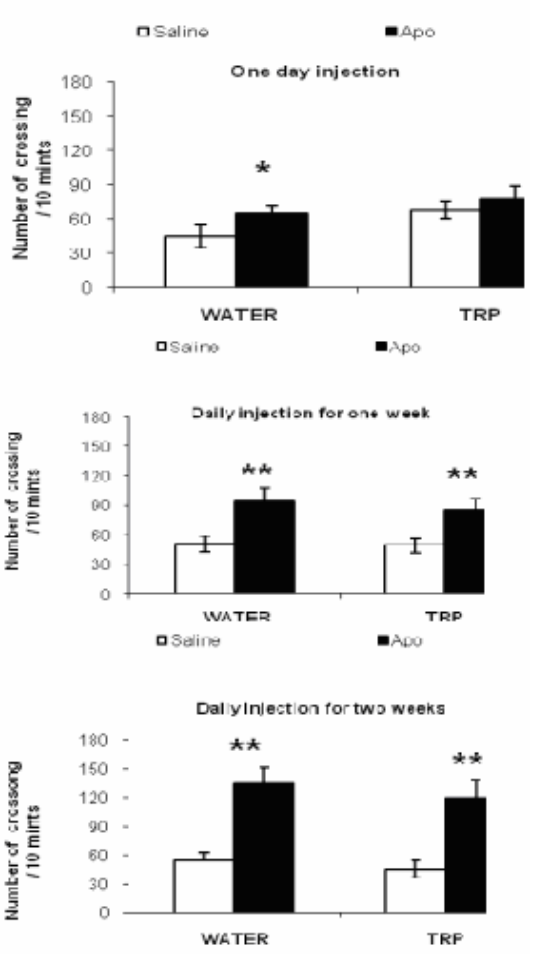

Fig. 1: Effects of administration of apomorphine (1.0 mg/kg) on number of crossing in activity box of rats pre-treated with tryptophan for two weeks. Values are means ± SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: *p<0.05, ** p<0.01 from respective saline injected controls animal following three-way ANOVA (repeated measures design).

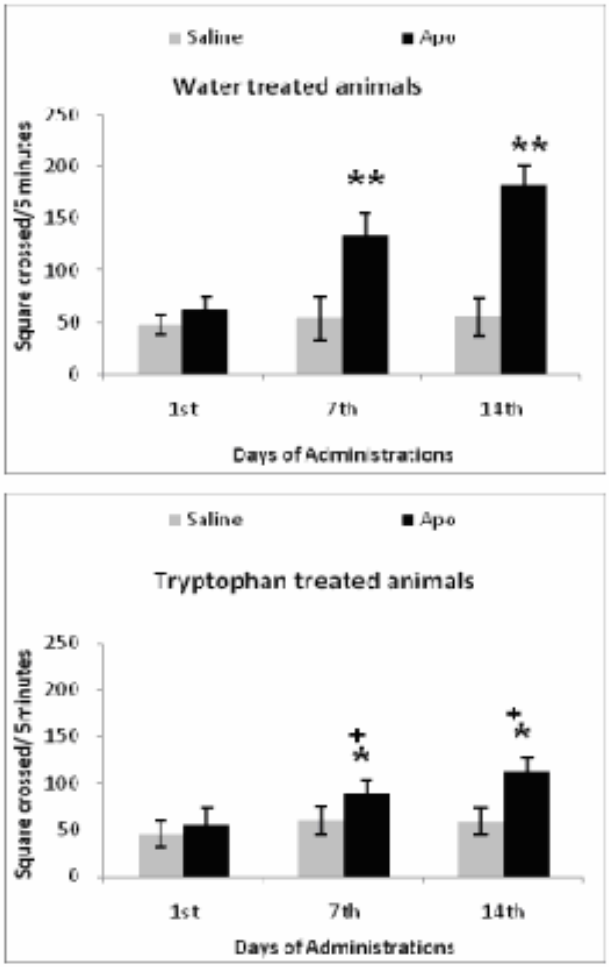

Fig. 2: Effects of administration of apomorphine (1.0 mg/kg) on number of square crossing in open field of rats pre-treated with tryptophan for two weeks. Values are means ± SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: *p<0.05, ** p<0.01 from respective saline injected controls animal, +p<0.01 from respective water treated animal following three-way ANOVA (repeated measures design).

of apomorphine suggesting sensitization. The sensitization effects of apomorphine were smaller in tryptophan treated animals.

Fig. 2 show effects of repeated administration of apomorphine at dose 1.0 mg/kg on activity (number of square crossed) in open field of rats pre-treated with tryptophan. Data analyzed by three-way ANOVA (repeated measures design) showed that effects of administration of apomorphine ($F=142.30$; $df= 1, 21$; $p<0.01$), tryptophan ($F=7.950$; $df= 1, 21$; $p<0.01$), repeated monitoring ($F=12.71$; $df= 2, 42$; $p<0.01$) were significant. Interaction between tryptophan, apomorphine and repeated monitoring ($F=0.688$; $df= 2, 42$) was not significant. Post-hoc analysis by Newman-Keuls test showed no significant change after one day administration of apomorphine in water as well as TRP treated animals. Administration of apomorphine increased activity after one week and two weeks treatment than single administration of apomorphine. Apomorphine –induced increased of activity were smaller in tryptophan treated animals.

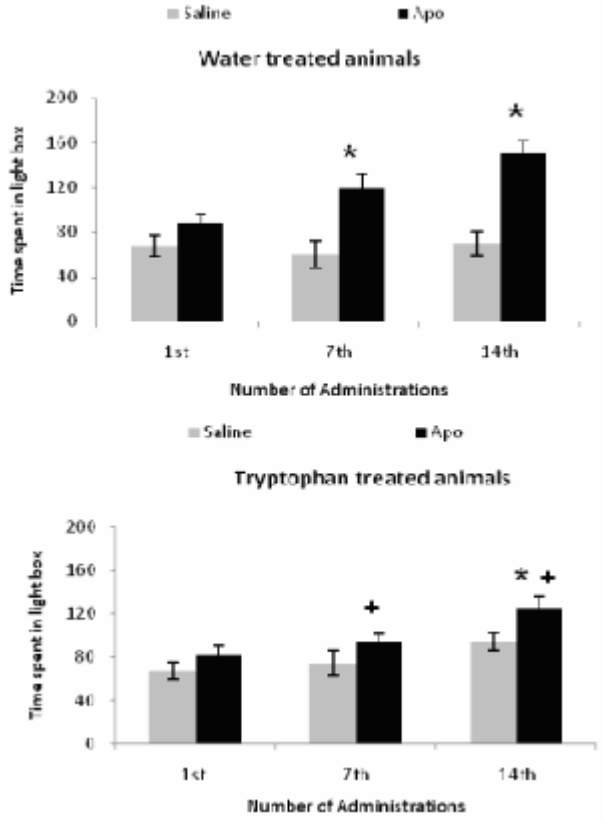

Fig. 3: Effects of administration of apomorphine (1.0 mg/kg) on activity in light dark box of rats pre-treated with tryptophan for two weeks. Values are means ± SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: * $p<0.01$ from respective saline injected controls, +$p<0.01$ from respective water treated animal following three-way ANOVA (repeated measures design).

Fig. 3 **s**how effects of repeated administration of apomorphine (14 days) on activity in light dark box of rats pre-treated with tryptophan. Data shows effects of repeated administration of apomorphine on time spent in light box of rats treated with tryptophan. Data analyzed by three-way ANOVA (repeated measures design) showed that effects of apomorphine ($F=87.716$; $df=1, 21$; $p<0.01$), repeated monitoring ($F=10.54$; $df=2, 42$; $p<0.01$) and interaction between tryptophan, apomorphine and repeated monitoring ($F=3.402$; $df=2, 42$; $p<0.05$) were significant but effects of tryptophan treatment ($F=0.989$; $df= 1, 21$; $p<0.01$) was not significant. Post-hoc analysis by Newman-Keuls test showed no significant change in activity of single administration in water as well as TRP treated animals. Administration of apomorphine increased activity (time spent in light box) in water as well as TRP treated animals and values were significantly greater after one week treatment in water treated animals. Values were significantly greater after two weeks treatment in water as well as TRP treated animals. Apomorphine-induced increased of activity were smaller in tryptophan treated animals and values were significantly smaller after two weeks treatment.

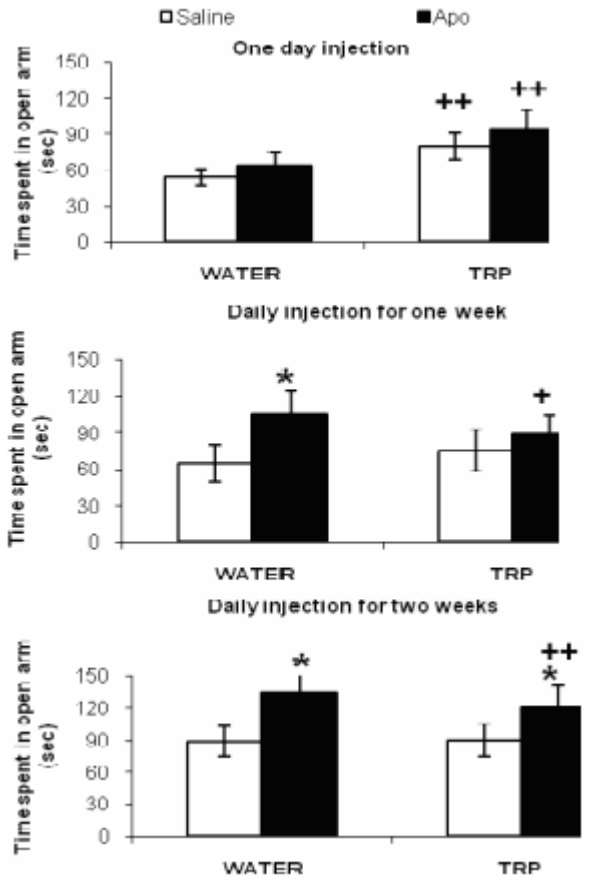

Fig. 4: Effects of administration of apomorphine (1.0 mg/kg) on activity in elevated plus maze of rats pre-treated with tryptophan for two weeks. Values are means

± SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: * $p<0.01$ from respective saline injected controls, +$p<0.05$, ++$p<0.01$ from respective water treated animal following three-way ANOVA (repeated measures design).

Fig. 4 **s**how effects of repeated administration of apomorphine at dose 1.0 mg/kg on activity in elevated plus maze of rats pre-treated with tryptophan. Data on time spent in open arm as analyzed by three-way ANOVA (repeated measures design) showed that effect of apomorphine ($F=161.08$; df=1, 21; $p<0.01$), repeated monitoring ($F=11.708$; df=2, 42; $p<0.01$), interaction between all factors ($F=14.23$; df=2, 42; $p<0.01$) as well as the effects of administration of tryptophan ($F=15.09$; df= 1, 21; $p<0.01$) were significant. Post-hoc analysis by Newman-Keuls test showed no significant change in activity on single administration in water but activity was increased in TRP treated animals. Apomorphine - induced increased activity were greater after one week and two weeks administration than single administration of apomorphine. Apomorphine induced activity were smaller in tryptophan than water treated animals and values were significantly smaller after two weeks treatment.

DISCUSSION

This preclinical study demonstrates the sensitization of apomorphine (1.0 mg/kg) on chronic treatment in the rats pre-treated with TRP (100mg/kg) for 2 weeks. Results suggests apomorphine induced motor activity were significantly higher in water treated over the tryptophan treated animals. Serotonin (5-HT) plays a central role not only in physiological responses but also regulates mood and behavior (Lucki. 1998). Altered level of brain serotonin is addressed to correspond with its precursor TRP, in the brain (Waltzing *et al.,* 1994), as inadequate supply of TRP can influence the level of serotonin in different regions of brain (Stancampiano *et al.,* 1997b; Lieben *et al.,* 2004). Apomorphine induced hyper locomotive effects were reduced in rats pretreated with tryptophan as results shown in light dark as well as in elevated plus maze models. It indicates that the involment of serotonin in the prevention of symptoms leads to psychotic syndrome and abuse potential produced with repeated administration of apomorphine (Ikram and Haleem, 2011) and other psycho stimulants (Fletcher *et al.,* 2008; Steiner *et al.,* 2010).

Present research reveals behavioral responses produced as result of repeated apomorphine administration was found attenuated in tryptophan pretreated animals. Like classical psycho- stimulant, acute administration of apomorphine raises motor behavior. Amphetamine and cocaine are also known to stimulate dopamine receptors indirectly, as they are shown to uplift synaptic level of dopamine, since apomorphine is D1/D2 receptor agonist so that it increases motor activity by affecting DA receptors directly (Haleem and Farhan, 2014). It has been addressed previously that tryptophan increases brain level of tryptophan and brain 5-HT as well and the level of 5-HT corresponds to the tryptophan level in the plasma (Haleem 1999). Results from the present study showed that apomorphine-induced hyper-locomotion activity was higher in familiar as well as in novel environment in water pre treated animals over the tryptophan pretreated animals. There are number of studies provides evidence of the sensitization on chronic treatment of apomorphine (Haleem *et al.,* 2005; Braga *et al.,* 2009a, 2009b; De Matos *et al.,* 2010; Dias *et al.,* 2010; Ikram and Haleem, 2011). Decreased 5-HT activity is reported to produce anxiolytic while increased 5-HT activity induces anxiogenic response. Stimulation of these receptors in raphe nuclei regulates the neuronal firing leads to drop the synaptic level of 5-HT and increase its metabolism. It is well known that apomorphine produces behavioral sensitization, characterized by a progressive increase in locomotive activity with repeated treatment (Mattingly *et al.,* 1997). Sensitization to apomorphine (1.0mg/kg) develops upon repeated administration, as assessed in an open field (Braga *et al.,* 2009). From previous studies it has been reported that apomorphine (0.5mg/kg) increased level of serotonin in different brain regions like striatum and hippocampus (Mendlin *et al.,* 1998). Bunney *et al.,* (1973) have reported that systemic administration of apomorphine inhibits DA neuronal impulse flow in the substantial nigra. It has been reported that DA turnover is decreased following the administration of apomorphine.

CONCLUSION

Present study concluded that treatment of tryptophan attenuate the sensitization induced by the apomorphine. Tryptophan administration for 2 weeks is shown to potentiate the adaptation to stress (Haleem *et al.,* 1998) which indicates the desensitized effect on somatodendritic 5-HT-1A auto receptors (Haleem 1999) beside this; chronic treatment of apomorphine causes the increased sensitivity of soma to dendritic 5-HT-1A receptors (Haleem *et al.,* 2005; Ikram and Haleem, 2011).

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