Reversal of haloperidol induced motor deficits in rats exposed to repeated immobilization stress

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Abstract: Stress is defined as a non specific response of body to any physiological and psychological demand. Preclinical studies have shown that an uncontrollable stress condition produces neurochemical and behavioral deficits. The present study was conducted to test the hypothesis that a decrease in the responsiveness of somatodendritic 5-hydroxytryptamine (5-HT)-1A receptors following adaptation to stress could attenuate haloperidol induced acute parkinsonian like effect. Results showed that single exposure (2h) to immobilization stress markedly decreased food intake, growth rate and locomotor activity but these stress-induced behavioral deficits were not observed following repeated (2h/day for 5 days) exposure of immobilization stress suggesting behavioral tolerance occurs to similar stress. An important finding of present study is a reversal of haloperidol-induced motor deficits in animals exposed to repeated immobilization stress than respective control animals. It is suggested that stress induced possible desensitization of somatodendritic 5-HT-1A as well as 5-HT-2C receptors could release dopamine system from the inhibitory influence of serotonin. On the other hand, an increase in the effectiveness of postsynaptic 5-HT-1A receptors elicits a direct stimulatory influence on the activity of dopaminergic neuron and is possibly involved in the reversal of haloperidol-induced parkinsonian like symptoms in repeatedly immobilized rats.

Keywords: Haloperidol, parkinsonism, repeated stress, serotonin, dopamine.

INTRODUCTION

Neuroleptic drugs are used in treatment of various neuropsychiatric disorders like schizophrenia (Ruess and Unsicker, 2001). Haloperidol, a typical neuroleptic is a dopamine (DA) receptor antagonist, used for the treatment of positive symptoms of schizophrenia (Perry, 1995). This drug block mesolimbic track of DA (Kaplan and Sadock, 1995). It is well documented that the mesolimbic pathway may have a role in regulating arousal, memory, stimulus processing, locomotor activity, and motivational behavior. Therefore, hyperactivity in this area causes over excitation of the different processes and also associated with the positive symptoms of schizophrenia (Ereshefsky and Lacombe, 1993).

Previous studies showed that due to blockage of DA neurotransmission, acute parkinsonism occur and if this condition sustained for longer period of time it results in super sensitivity of DA receptors (Jan et al, 1990; Jenner and Marseden, 1998). Experimental evidence suggested that serotonergic systems are involved in the modulation of dopaminergic neurons. In this regard, serotonergic mechanisms are of particular importance both in view of their modulation of dopaminergic transmission and their key role in the control of mood, cognition, and motor behavior (Millan, 2000). Multiple receptors of serotonin (5-HT) play important role in some pathological and psychophysiological conditions. 5-HT1A receptors are involved in the etiology of schizophrenia (Newman-Tancredi et al., 1998).

A stressful stimulus has been used to produce learned helplessness in different animal models of depression. It was suggested that adaptive changes produce in response to repeated exposure to an uncontrollable stressor (Kennet et al., 1987; Haleem and Parveen 1994; Haleem et al., 1988). Serotonergic responses to stress are also part of this mechanism. It was therefore suggested that change in receptor responsiveness is possibly involved in adaptation to stress. In subsequent studies it has shown that adaptation to repeated restraint stress is possibly associated with the decrease in the effectiveness of soma to dendritic 5-HT-1A receptors (Haleem, 1999). On the other hand, stimulation of somatodendritic 5-HT-1A receptors decrease the availability of 5-HT at 5-HT-2C receptors in the striatum could release DA neurotransmission from the inhibitory influence of 5-HT to produce antiparkinsonian effects (Shireen and Haleem, 2011; Haleem et al., 2004).

The present study was designed to test the hypothesis that a possible decrease in the effectiveness of soma to dendritic 5-HT-1A receptors following adaptation to stress could attenuate and/or reverse the haloperidol induced acute parkinsonian like effect. The mechanism involved in the attenuation of haloperidol induced EPS following adaptation to stress is discussed.

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MATERIAL AND METHODS

Animals
Locally 32 bred male Albino-Wistar rats weighing about 150-200g were caged individually in specifically designed cages with saw-dust covered floor, in a quiet room with a free access to water and rodent diet for at least 3 days before starting the experiment, so that the rats could adapt to the novel environment.

Drugs and their Administration
All chemicals and drugs used during the study were purchased from Sigma Chemical Company (USA), Research Biochemical (RBI, USA), G.D. Searle LLC. U.S.A. Haloperidol was injected intraperitoneal at concentration of 1mg/kg in saline (0.9%, w/v, NaCl) while control rats received 1ml/kg saline.

Experimental Protocol
Thirty two animals were weighed and randomly divided into two groups of sixteen animals: (i) Unrestrained (ii) Restrained. Food intake and body weight of the rats were monitored daily. Weighed amount of food was placed in the food bucket of each animal immediately before the injection. Animals of the stressed groups were immobilized by taping their legs to wire grids. After release from the grids, animals of stressed groups were also kept back in their home cages with free access to food and water. Cumulative food intake and body weight changes were monitored next day between 8:00 and 9:00 h. Exploratory activity in a familiar (home cage activity) and novel environment (open field) and impairment of motor activity on Rota-Rod were monitored, in a balanced design, between 9:00 and 12:00 h following first and 5th day of stress. On 6th day of stress, animals were further divided into two groups (i) saline (0.9%NaCl) (1 ml/Kg) injected (ii) haloperidol (1mg/kg) injected animals. Behavioral activities in familiar (home cage activity) and novel environment (open field) and impairment of motor activity on Rota-Rod were monitored, in a balanced design, between 9:00 and 12:00 h following saline and haloperidol injections.

Restraining Procedure
The animals were restrained on wire grids of 10"×9" fitted with a Perspex plate of 9"×6.5". Immobilization was produced by pressing the fore legs of the rats through the gaps in the metal grids and taping them together with Zinc Oxide plaster tape. Hind limbs were also taped and the head of the animal rested on the Perspex plate. After 2-h of restraining period the animals were released and return to their home cage.

Behavioral method
Open Field Activity Test
The assessment of locomotor activity and exploration in a novel environment was performed in an open field. In this test, motor activity of rat measures in an open novel space, from which escape is prevented by a surrounding wall. The open field apparatus used in the present investigation consisted of a square area 76x76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine the activity a rat was placed in the center square of the open field. The numbers of squares crossed with all four paws were scored for 5 min.

Motor Coordination
Motor coordination was assessed on a Rota-rod (UGO-BASILE, Italy). The Rota-rod (Knurled Perspex) with a drum of 7 cm radius and a speed of 2-20 revolutions/ min during training session and a fixed speed of 20 revolutions/min during test session. A day before the treatment rats were trained in a single session until they attained 150 sec on Rota-rod. The latency to fall in a test session of 150 sec was taken as measure of motor coordination.

Home Cage Activity Test
The assessment of locomotor activity and exploration in a familiar environment as it may be altered by drug administration was done by home cage activity test. The apparatus used in this study was a rectangular Perspex activity cage consisted of small square area (26x26x26 cm) with sawdust-covered floor. Testing was performed in a quiet room under white light. Before monitoring the activity an animal was placed in it for 15 minutes for habituation. Numbers of crossings across the box were monitored for 10 min.

STATISTICAL ANALYSIS
Results are represented as mean ± S.D. Behavioral data was analyzed by ANOVA. Individual comparisons were made by Newman-Kuels test.

RESULTS
Fig. 1 shows the effect of single (2h) and repeated (2h/day for 5 days) immobilization stress on daily changes of food intake in rats. Two way ANOVA revealed significant effects of stress (F=181.04 p<0.01 df 1,120) and days (F=64.82 p<0.01 df 4,120). The interaction between stress and days were also significant (F=4.08 p<0.01 df 4, 120). Post hoc analysis by Newman Kuels test showed that exposure to first episode of 2 h immobilization stress on day 1 to saline injected animals produced a pronounced decrease in 24 h cumulative food intake. The decreases were attenuated following 2nd and 3rd, 2 h/day, immobilization and did not occur following 4th and 5th day of immobilization stress suggesting adaption to stress in saline injected animals.

Fig. 2 shows the effect of single (2h) and repeated (2h/day for 5 days) immobilization stress on daily changes of body weight in rats. Two way ANOVA revealed significant effects of stress (F=23.96 p<0.01 df 1,120) and
days (F=80.65 p<0.01 df 4, 120). The interaction between stress and days was also significant (F=85.07 p<0.01 df 4, 120). Post hoc analysis by Newman-Kuels test showed that exposure to first episode of 2 h immobilization on day 1 to animals produced a pronounced decrease in body weight. The decreases were attenuated following 2nd and 3rd, 2 h/day, immobilization and did not occur following 4th and 5th immobilization suggesting adaptation to the stress schedule.

Fig. 1: Effects of single (2h) and repeated (2h/day for 5 days) immobilization stress on 24h cumulative daily food intake and body weight in rats. Significant differences by Newman-Keuls test. *p<0.01 from respective unstressed rats.

Fig. 2: Effects of single (2h) and repeated (2h/day for 5 days) immobilization stress on daily changes of body weight in rats. Values are means ±S.D. (n=16). Significant differences by Newman-kuels test. *p<0.05 and **p<0.01 from respective unstressed animals.

Fig. 3 shows effect of single (2h) and repeated (2h/day for 5 days) restraint stress on locomotor activity (in an activity box) in rats. Two way ANOVA revealed significant effects of stress (F=54.37 p<0.01 df 1, 60) and days (F=6.42 P<0.01 df 1,60) but interaction between stress and days was non significant (F=3.21 NS). Post hoc analysis by Newman kuels test revealed a marked decrease in exploratory activity was observed in rats exposed to first episode of 2h immobilization on day 1 as compare to repeated immobilization stress on day 5.

Fig. 4 shows changes of motor activity in animals injected with saline or haloperidol (1mg/kg) and exposed to repeated immobilization (2h/day for 5 days) stress. Two way ANOVA revealed significant effect of haloperidol (F=39.538 p<0.01 df 1, 28), stress (F=31.711 p<0.01 df 1, 28) and interaction between haloperidol and stress (F=8.9171 p<0.01 df 1, 28). Post hoc analysis showed that acute administration of haloperidol at a dose of 1 mg/kg significantly decreased locomotor activity in unstressed animals but these haloperidol-induced motor deficits were attenuated following repeated exposure to immobilization stress (2h/day for 5 days).

Fig. 4A and 4B: Effects of repeated (2h/day for 5 days) immobilization stress on haloperidol (1mg/kg) induced deficits of exploratory activity (in an open field) in rats. Values are ± SD (n=8) of unrestraint and restraint rats. Significant differences by Newman-Keuls test. +p<0.01 from respective saline injected rats, *p<0.01 from respective unrestraint rats following two-way ANOVA.

Fig. 5 shows effect of single (2h) and repeated (2h/day for 5 days) restraint stress on locomotor activity (in an activity box) in rats. Two way ANOVA revealed significant effects of stress (F=54.37 p<0.01 df 1, 60) and
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days (F=88.94 p<0.01 df 1, 60). The interaction between stress and days was not significant (F=1.31 N.S. Post hoc analysis showed that locomotor activity was decreased in rats exposed to first episode of 2h stress following single and repeated immobilization stress than respective unstressed animals. The decreases were more pronounced after first episode of stress as compare to repeated immobilization in rats.

Post hoc analysis showed that administration of haloperidol at a dose of 1mg/kg significantly decreased locomotor activity in unstressed animals. On the other hand, haloperidol-induced motor deficits were attenuated in rats exposed to repeated immobilization stress (2h/day for 5 days).

**Fig. 5**: Effects of single (2h) and repeated (2h/day for 5 days) immobilization stress on locomotor activity (in an activity box) in rats. Significant differences by Newman-Keuls test. *p<0.01 from respective 1st day unrestrained/restrained animals. +p<0.01 from respective unrestrained animals.

**Fig. 6**: Effect of repeated (2h/day for 5 days) immobilization stress on haloperidol (1mg/kg) induced locomotor activity (in an activity box) in rats. Significant differences by Newman-Keuls test. *p<0.01 from respective unstressed animals. +p<0.01 from respective saline injected animals.

**Fig. 7**: Effects of single (2h) and repeated (2h/day for 5 days) immobilization stress on haloperidol (1mg/kg) induced deficits of locomotor activity (in an activity box) in rats. Two way ANOVA revealed significant effect of haloperidol (F=39.53 p<0.01 df 1, 28), stress (F=31.71 p<0.01 df 1, 28) and interaction between haloperidol and stress (F=8.91 p<0.01 df 1, 28). Post hoc analysis showed that administration of haloperidol at a dose of 1mg/kg significantly decreased locomotor activity in unstressed animals. On the other hand, haloperidol-induced motor deficits were attenuated in rats exposed to repeated immobilization stress (2h/day for 5 days).

**Fig. 8**: Effects of repeated (2h/day for 5 days) immobilization stress on haloperidol-induced deficits of motor coordination on Rota-Rod in rats. Values are ± SD (n=16) of unrestrained and restrained rats. Significant differences by Newman-Keuls test. *p<0.01 from respective unstressed rats, +p<0.01 from respective saline injected rats following two-way ANOVA.
external environment in form of stimuli such as heat, pain tumors and cold, loudness or may originate within body in form of uncontrollable stress (Weiss et al., 2013). The present study showed that single (2h) exposure to immobilization stress produced greater decrease in cumulative daily food intake and body weight changes and these behavioral deficits were not observed following repeated exposure (2h/day for 5 days) to stress (figs. 1 and 2). The present data on the effects of immobilization on cumulative daily food intake and body weight changes is consistent to support the notion that behavioral tolerance/adaptation to a stress schedule develops when the animals are exposed to the same stressor repeatedly (Haleem and Parveen, 1994; Haleem, 1999; Haleem et al., 2007; Haleem et al., 2013).

DISCUSSION

The present study was designed to test the hypothesis that a decrease in the responsiveness of soma to dendritic 5-HT1A receptors following adaptation to stress could attenuate haloperidol induced acute parkinsonian like effect. It was found that single exposure (2h) to immobilization stress markedly decreased food intake, growth rate and locomotor activity but these stress-induced behavioral deficits were not observed following repeated (2h/day for 5 days) exposure of immobilization stress. An important finding of present study is a reversal of haloperidol-induced motor deficit in animals exposed to repeated immobilization stress than respective control animals.

Stress is a feature of all lives and it can lead to physical and mental illnesses including depression, nervous breakdown and cardiac failure. It act as predisposing and precipitating factor in the onset of affecting illness especially depression (Brown et al., 1987). Preclinical studies have shown that neurochemical alterations as well as behavioral deficits were produced following episodes of uncontrollable stress (Weiss et al. 1981; Haleem et al., 2007; Haleem et al., 2013). Stress may elicits from external environment in form of stimuli such as heat, cold, loudness or may originate within body in form of stimuli such as high blood pressure, pain tumors and unpleasant thoughts (Tortora and Anagnostakos, 1990).

Stress induced behavioral deficits was observed in previous studies include marked decrease in food intake, growth rate and locomotion (Kennett et al., 1985; Haleem et al., 1998, Haleem et al., 2013). The present study showed that single (2h) exposure to immobilization stress produced greater decrease in cumulative daily food intake and body weight changes and these behavioral deficits were not observed following repeated exposure (2h/day for 5 days) to stress (figs. 1 and 2). The present data on the effects of immobilization on cumulative daily food intake and body weight changes is consistent to support the notion that behavioral tolerance/adaptation to a stress schedule develops when the animals are exposed to the same stressor repeatedly (Haleem and Parveen, 1994; Haleem, 1999; Haleem et al., 2007; Haleem et al., 2013).

Fig. 8 shows effect of single (2h) and repeated (2h/day for 5 days) immobilization stress on haloperidol (1mg/kg) induced deficits of motor coordination on Rota-rods in rats. Two way ANOVA revealed significant effects of haloperidol (F=47.04 p<0.01 df 1, 28) and interaction between stress and haloperidol (F=61.79 p<0.01 df 1, 28). Effect of stress (F=0.208 N.S) was not significant. Post hoc analysis showed that exposure to immobilization stress significantly impaired motor coordination in rats. Administration of haloperidol impaired motor coordination in unstressed animals. Haloperidol induced impaired motor coordination was reversed in rats repeatedly exposed to immobilization stress.

Several lines of evidence have clearly demonstrated that 5-HT is involved in stress responses (Haleem and Parveen, 1994; Haleem, 2011; Haleem et al., 2013). Stress is linked to the central 5-HT system and thought to be a part of a coping such response involving several 5-HT receptors. Evidence has accumulated that 5-HT turnover is enhanced by following exposure to various stressors including exercise and foot shock although brain levels of 5-HT are not always altered (Andrew and Matthews, 2004; Bebbington et al., 2003; Chaouloff et al., 1999; Kennett and Joseph, 1981). It has been also shown that stress-induced increases of brain serotonin are caused by an increase in the availability of tryptophan (Kennett and Joseph, 1981), the precursor of 5-HT, or an increase in the activity of tryptophan hydroxylase (Dunn, 2000; Haleem and Parveen, 1994; Singh et al, 1990) the rate limiting enzyme of 5-HT biosynthesis. Microdialysis studies showed an increase in extracellular levels of serotonin (Adell et al, 1997; Fujino et al, 2002; Shimizu et al, 1992) in different areas of the brain following exposure to different types of stressors. Preclinical studies show that raphe-hippocampal serotonergic neurotransmission and regulation particularly through 5-HT1A receptors can explain vulnerability or resistance to stress stimuli (Haleem et al. 2007b; Haleem, 2011). An effective...
feedback mechanism could control the availability of 5-HT at terminal and postsynaptic sites. 5-HT-1A receptors are located on the soma and dendrites of serotonergic neurons whereas 5-HT-1B receptors located on the nerve terminal end are the mediator of the control mechanism. An increase in the availability of 5-HT in the terminal region following a decrease in the effectiveness of both these receptors have been reported to be involved in adaptation to repeated restraint stress (Haleem, 1996). On the other hand, an increase in the effectiveness of these receptors was observed in ethanol treated rats (Haleem et al. 2007a) impaired adaptation in repeatedly restraint rats (Balsara et al. 1979). Furthermore, a decrease in the responsiveness of somatodendritic 5-HT-1A receptor was also involved in the reversal of haloperidol induced tardive dyskinesia (Shireen, and Haleem, 2011). However, it is difficult to explain the attenuation of haloperidol induced parkinsonian like effects in rats adapted to repeatedly immobilized stress as observed in the present study in terms of decrease in the responsiveness of somatodendritic 5-HT-1A receptors. It is expected that a decrease in the effectiveness of somatodendritic 5-HT-1A receptors would release more 5-HT at 5-HT-2C receptors site and therefore increases the inhibitory effects of 5-HT on the dopamine neurotransmission.

It has been reported that the serotonergic system could regulate the activity of dopaminergic neurons. The nature of this regulation is thought to be inhibitory at the somatodendritic region of DA system in the mid brain as well as in the terminal region (Sandyk, and Fisher 1988; Samad and Haleem, 2009; Shireen And Haleem, 2011). Administration of 5-HT-2C receptor antagonist such mianserin and mesulergine could release DA neurotransmission from the inhibitory influence of 5-HT to attenuate acute parkinsonian like effects of typical neuroleptics (Shireen and Haleem, 2011). Studies have shown a change in the responsiveness of 5-HT-2C receptors following adaptation to repeated stress (Haleem et al., 2004) and long-term administration of selective serotonin reuptake inhibitor (SSRI). The present results can partly be explained in terms of a decrease in the effectiveness of 5-HT-2C receptor in rats adapted to repeated immobilization stress resulting in an extra decrease in the inhibitory influence of 5-HT on DA transmission. Thus desensitization of soma to dendritic 5-HT-2C receptor in rats adapted to repeatedly immobilization resulted in the reversal of haloperidol induced parkinsonism.

Unlike 5-HT-2C receptors, stimulation of postsynaptic 5-HT-1A receptors may lead to an increase in the activity of dopaminergic neurons is also shown in neurochemical investigation (Haleem et al. 2004). It has also been reported that supersensitive postsynaptic 5-HT-1A receptors following exposure to an uncontrollable stressor is involved in antidepressant or adaptive effects (Haleem, 1996). The present results on the reversal of haloperidol induced parkinsonian like effects can also be explained in terms of increased effectiveness of postsynaptic 5-HT-1A receptors that would be expected to produce direct stimulatory influence on the activity of dopaminergic neurons by inhibiting haloperidol induced DA D2 receptor blockade.

CONCLUSION

The present study showed that single exposure (2h) to immobilization stress markedly decreased food intake, growth rate and locomotor activity but these stress-induced behavioral deficits were not observed following repeated (2h/day for 5 days) exposure of immobilization stress suggesting behavioral tolerance occurs to similar stress. An important finding of present study is a reversal of haloperidol-induced motor deficits in animals exposed to repeated immobilization stress than respective control animals. It is suggested that serotonin has an inhibitory as well as stimulatory influence on the activity of dopamine system. Stress induced possible desensitization of soma to dendritic 5-HT-1A as well as 5-HT-2C receptors could release dopamine system from the inhibitory influence of serotonin. On the other hand, an increase in the effectiveness of postsynaptic 5-HT-1A receptors elicits a direct stimulatory influence on the activity of dopaminergic neuron and is possibly involved in the reversal of haloperidol-induced parkinsonian like symptoms in repeatedly immobilized rats.

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