ORIGINAL ARTICLE

SEROTONIN-1A RECEPTOR RESPONSIVENESS IN STRESS AND FOLLOWING ADAPTATION TO STRESS

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ABSTRACT

Stress is one of the environmental factors that may predispose psychiatric illness such as, depression. Stress may come from external environment in the form of stimuli such as heat, cold, loud noise and lack of oxygen. A deficiency of serotonin (5-hydroxytryptamine; 5-HT) is described in human depression. Parallel studies on experimental animals show that exposure to an uncontrollable stress inducing situation elicits behavioral deficits and increases serotonin metabolism in the brain. Stress-induced behavioral deficits and the increases of brain serotonin did not occur when the stress was administered repeatedly for 5 days, suggesting adaptation has occurred. The present study shows that responses to 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT), a selective 5-HT 1A agonist decreased following exposure to single stress and the decreases were normalized following adaptation to stress. The drug 8-OH-DPAT was also found to attenuate stress-induced behavioral deficits. The results are discussed in the context of stress-induced psychiatric disorder such as, depression and its treatment by 5-HT 1A agonist.

Keywords: Stress, depression, serotonin adaptation to stress, 8-OH-DPAT.

INTRODUCTION

Stress is a feature of all lives. It acts as predisposing and precipitating factor in onset of affecting illness specially depression (Brown *et al*., 1987). Studies on experimental animals show that an uncontrollable stress produces neurochemical changes and behavioral deficits (Weiss *et al*., 1981). An episode of stress increases brain serotonin (5-hydroxytryptamine; 5-HT) metabolism and precipitate behavioral deficits comparable to a model of depression (Haleem, 1999). Behavioral adaptation to stress schedule develops, when the same stress is administered repeatedly (Haleem and Parveen, 1994). Similarly, increases of brain 5-HT metabolism do not occur in animals adapted to repeated stress schedule (Haleem and Parveen, 1994).

A dysregulation of activity at the levels of 5-HT 1A receptor is implicated in the pathophysiology of anxiety, depression and other psychotic disorders (Maes and Meltzer, 1995). 5-HT 1A receptors are present on the soma and dendrites of 5-HT neurons and also on postsynaptic neurons in various region of the limbic system (Verge *et al*., 1985). The stimulation of somatodendritic receptor inhibits electrical activity in serotonergic neurons (Blier and De Montigny, 1987; Blier and Ward, 2003), synthesis and release of 5-HT from nerve ending is also decreased (Horth and Magnusson, 1988; Adell *et al*., 1991). A desensitization of 5-HT 1A presynaptic receptor (Haleem, 1999) and sensitization of 5-HT 1A postsynaptic receptor (Haleem *et al*., 2002) occur following adaptation to stress.

The prototypical 5-HT 1A agonist 8-OH-DPAT exhibits anxiolytic (Carli and Samanin, 1988; Remy *et al*., 1996) and antidepressant-like (Kennett *et al*., 1987; Martin *et al*., 1990) properties in animal models. Acute administration of 8-OH-DPAT stimulates presynaptic 5-HT 1A receptor to decreases 5-HT synthesis and turnover (Haleem, 1990) and postsynaptic receptors to produce hyperactivity syndrome (Haleem, 1992). Repeated administration of 8-OH-DPAT decrease responsiveness of somatodendritics 5-HT 1A autoreceptor (Blier and De Montigny, 1987).

The present study is designed to monitor the effects of prior administration of 8-OH-DPAT on restraint-induced behavioral deficits and adaptation to repeated restraint stress in rats.

MATERIALS AND METHODS

Animals

Twenty four locally bred male albino Wistar rats weighing 200-220 purchased from The Agha Khan University, Pakistan were housed individually under a 12-hours light and dark cycle (light on at 0600 hours) with free access to cubes of standard rodent diet and tap water 3 days before experimentation. All experiments were performed according to a protocol approved by the local Animal Care Committee.

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EXPERIMENTAL PROTOCOL

In the beginning of experiment 24 animals were divided equally into saline (1ml/kg) and 8-OH-DPAT (0.25 mg/kg) treated animals. The animals received respective treatment daily till the end of the experiment.

After 3 days of pre-treatment animals were further divided into unrestrained and restrained sub-groups. Animals of restrained groups were restrained for 2 h daily between 11:30 to 13:30 for 5 days. The animals assigned for unrestrained groups were left to their home cages. 24 h cumulative food intake and body weights were monitored daily. Light-dark activity (for 5 minutes) was monitored from 08:00 to 10:00 h before injections following 1st, 3rd and 5th restraint stress.

Restraining the animals

The animals were restrained on wire grids of 10″x9″ fitted with a Perspex plate of 9″x6.5″. Restraining procedure was same as described earlier (Haleem and Parveen, 1994; Samad *et al*., 2005). Immobilization was affected 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 animal rested on the Perspex plate. After 2h of restraining period the animals were released and returned to their home cage.

Light-dark activity test

A test was conducted in a locally made two-compartment box. The compartment of equal size (26x26x26 cm), with an access (12x12 cm) 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 dark compartment. To determine the activity, a rat was placed in the light compartment of the box. Time spent in the light compartment was monitored for a cut off time of 5 min.

STATISTICAL ANALYSIS

Data on pre-stress changes of food intake and body weight was analyzed by Student's t-test. Data on the effect of 8-OH-DPAT on daily changes of food intake, body weight and light-dark activity following single and repeated restraint stress was analyzed by three-way ANOVA. Post-hoc analysis was done by Newman-Keuls test: p values < 0.05 were taken as significant.

RESULTS

Figure 1 shows the effects of two (one daily) injections of 8-OH-DPAT (0.25 mg/kg) on food intake and body weight. Student's t-test showed that differences between food intake and body weight were not significant for the two groups.

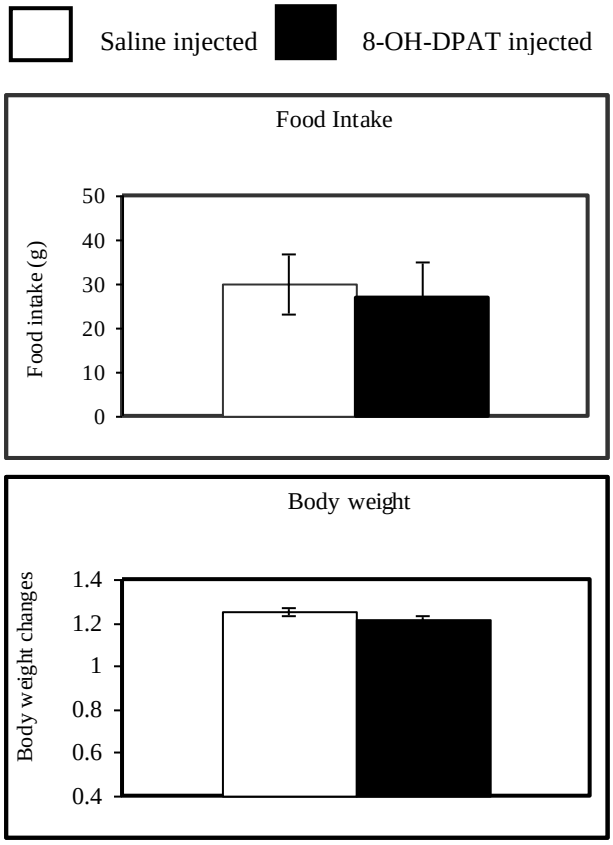

Fig. 1: The effect of two (one daily) injections of 8-OH-DPAT (0.25 mg/kg) on food intake and body weight changes. Values are means + S.D. (n=12).

Figure 2 shows the effects of single (2 h) and repeated (2 h/day for 5 days) restraint stress on 24 h cumulative food intake in saline and 8-OH-DPAT treated rats. Three-way ANOVA showed significant effect of stress ($F=181.56$ $df=1,100$ $p<0.01$). Effects of day ($F=2.92$ $df=4,100$ $p>0.05$) and administration of 8-OH-DPAT ($F=2.15$ $df=1,100$ $p<0.01$) were not significant. Interaction between stress*8-OH-DPAT ($F=5.20$ $df=4,100$ $p<0.05$) and stress*day ($F=13.01$ $df=4,100$ $p<0.01$) were significant. Interaction between day*8-OH-DPAT ($F=1.67$ $df=4,100$ $p>0.05$) and day*stress*8-OH-DPAT ($F=1.91$ $df=4,100$ $p>0.05$) were not significant. Post-hoc analysis showed that saline as well as 8-OH-DPAT injected rats exposed to 2h/day restraint stress exhibited a decrease in food intake following 1st day. The decreases attenuated following 2nd and 3rd (2 h/day) restraint were not significant after the 4th and 5th day (2 h/day) restraint. First day restraint-induced decreases of food intake were smaller in 8-OH-DPAT than saline treated rats.

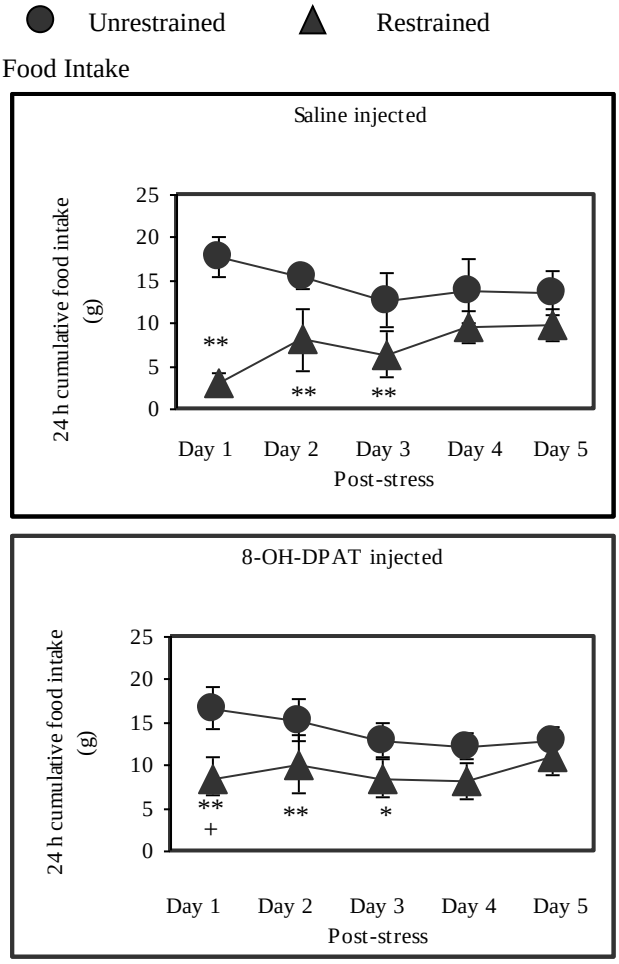

Fig. 2: Effects of daily (2h/day) restraint stress for 5 days on daily changes of food intake in animals treated with saline and 8-OH-DPAT. Values are means + S.D. (n=6). Significant differences by Newman-Keuls test: **p<0.01 and *p<0.05 from respective day saline or 8-OH-DPAT injected unrestrained rats, +p<0.01 from saline injected restrained rats following Three-way ANOVA.

Figure 3 shows the effects of single (2 h) and repeated (2h/day for 5 days) restraint stress on 24 h changes in body weights of saline and 8-OH-DPAT treated rats. Three-way ANOVA showed significant effects of day ($F=23.36$ df=4,100 $p<0.01$), stress ($F=182.34$ df=1,100 $p<0.01$) and 8-OH-DPAT administration ($F=6.27$ df=1,100 $p<0.01$). Interaction between stress*8-OH-DPAT ($F=15.24$ df=4,100 $p<0.01$), day*8-OH-DPAT ($F=4.13$ df=4,100 $p<0.01$) and stress*day ($F=32.74$ df=4,100 $p<0.01$) were significant. Interaction between stress*day*8-OH-DPAT ($F=3.62$ df=4,100 $p>0.05$) was not significant. Post-hoc analysis showed that saline injected rats exposed to 1st day 2 h/day restraint-stress exhibited a decrease in body weight. The decreases were attenuated following 2nd and 3rd (2 h/day) restraint were not significant following 4th and 5th day (2 h/day) restraint. In 8-OH-DPAT treated animals, single (2 h) restraint stress decreased body weights on day 1 and day 2, the decreases on following days were not significant. First and 2nd (2 h/day) restraint decreased weight more in saline than 8-OH-DPAT treated animals.

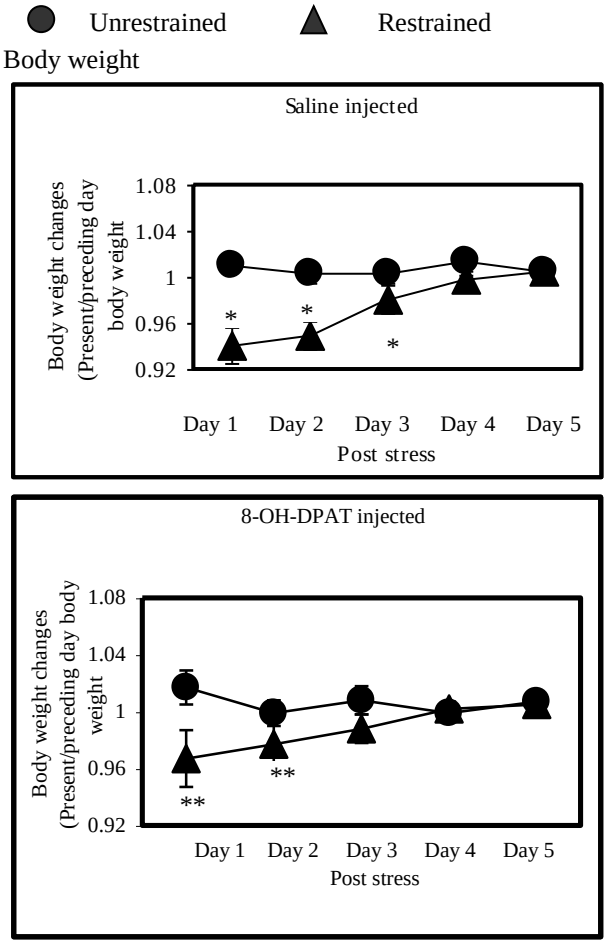

Fig. 3: Effects of daily (2h/day) restraint stress for 5 days on daily changes of body weight of animals treated with saline and 8-OH-DPAT. Values are means + S.D. (n=6). Significant differences by Newman-Keuls test: *p<0.01 from respective day saline or 8-OH-DPAT injected unrestrained rats, +p<0.01 from saline injected restrained rats following Three-way ANOVA.

Figure 4 shows the effects of 2 h restraint stress on time spent in light box (light-dark activity) in saline and 8-OH-DPAT injected animals, following 1st, 3rd and 5th day, 2h/day restraint stress. Three-way ANOVA showed that significant effect of day ($F=16.43$ df=1,60 $p<0.01$), stress ($F=9.49$ df=1,60 $p<0.01$) and 8-OH-DPAT administration ($F=25.47$ df=1,60 $p<0.01$). Interaction between day*stress ($F=2.60$ df=2,60 $p>0.05$), stress*8-OH-DPAT ($f=3.25$ df=2,60 $P>0.05$), day*8-OH-DPAT ($f=1.61$ df=2,60 $P>0.05$) and stress*day*8-OH-DPAT ($F=0.73$ df=2,60 $p>0.05$) were not significant. Post-hoc analysis showed that an episode of 2 h restraint decreased time spent in light box in saline as well as 8-OH-DPAT treated animals. 8-OH-DPAT treated unrestrained and restrained animals

spent greater time spent in light compartment than saline treated unrestrained and restrained animals following 1st and 3rd day (2 h/day) restraint.

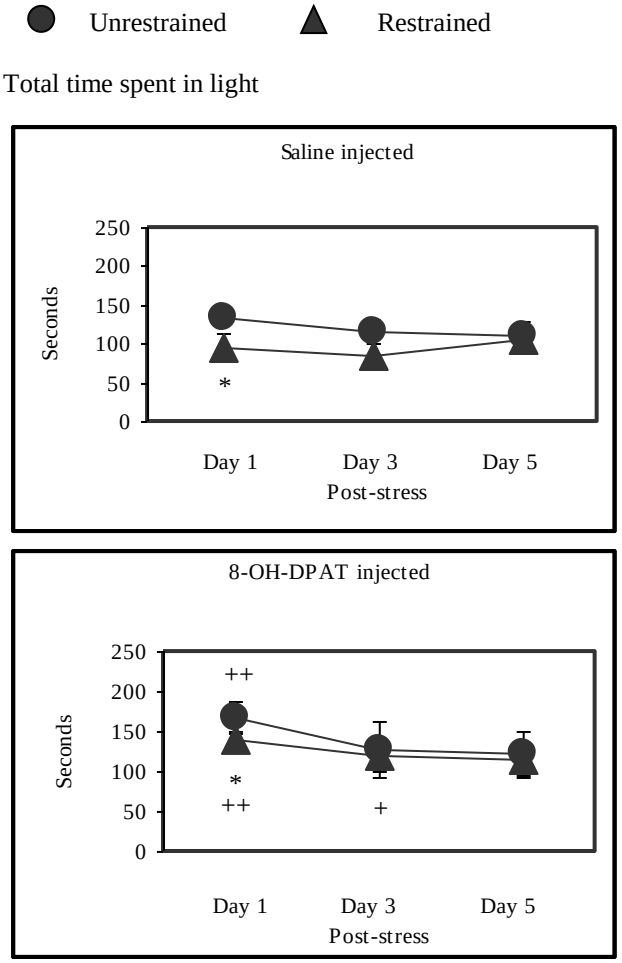

Fig. 4: Light-dark activity in repeated saline and repeated 8-OH-DPAT injected unrestrained and restrained animals. Values (means + S.D. n=6) are 24 h after the 1st, 3rd or 5th (2h/day) restraint stress. Significant differences by Newman-Keuls test: *p<0.05 from respective saline or 8-OH-DPAT injected unrestrained rats, +p<0.05 and ++p<0.01from saline injected rats following Three-way ANOVA.

DISCUSSION

Previously it has been shown that an episode of 2 h restraint stress decreased 24 h cumulative food intake, growth rate and locomotor activity (Haleem and Parveen, 1994; Samad *et al*., 2005). The deficits were not observed, when rats were restrained 2 h/day for 5 days. It was suggested that adaptation to stress schedule occur when similar type of stress is administered repeatedly (Haleem *et al*., 1988; Haleem and Parveen, 1994). In the present study, the effects of restraint on 24 h changes of cumulative food intake are consistent with the previous studies (Haleem *et al*., 1988; Haleem and Parveen, 1994; Samad *et al*., 2005). Present data show that food consumption of unrestrained rats treated with saline and 8-OH-DPAT is decreasing up to 3 days (fig. 2). The decreases may be due to stress of the injection but later on it became constant.

Previously it has been reported that 8-OH-DPAT and other 5-HT 1A agonist attenuate the anorexia and body weight loss caused by immobilization stress (Dourish *et al*., 1986; Shimizu *et al*., 2000). In the present study prior administration (3 days) of 8-OH-DPAT attenuated restraint-induced decreases in food intake (fig. 2) and growth rate (fig. 3). These decreases were normalized following 5th (2 h/day) restraint stress. It is also observed that single restraint-induced decreases were smaller in 8-OH-DPAT than saline treated animals.

From fig. 3 it appears that 8-OH-DPAT attenuate anxiogenic effect of stress in light-dark transition test (anxiety model), which is consistent with the previous studies (Romanuik *et al*., 2001). They have reported administration of 8-OH-DPAT (0.2 mg/kg) into the dorsal raphe nucleus increased time spent in lit compartment. The authors have argued that 8-OH-DPAT acts on presynaptic 5-HT 1A receptor to decrease fear behavior. The present study shows that anxiogenic effects of stress smaller in 8-OH-DPAT injected animals.

Single administration of 8-OH-DPAT stimulates presynaptic 5-HT 1A receptor and decreases 5-HT metabolism and synthesis rate (Hjorth *et al*., 1982; Sharp *et al*., 1989), while these responses to 8-OH-DPAT are decreased to repeatedly restraint adapted rats (Haleem and Parveen, 1994). Blier and De Montigny (1987) have reported that long term (2 weeks) administration of 5-HT 1A agonist (8-OH-DPAT & gepirone) desensitized somatodendritic 5-HT 1A autoreceptor whereas postsynaptic 5-HT 1A receptors in the hippocampus remain normosensitive.

Investigation on the serotonergic mechanism in adaptation to stress show that a decrease in the responsiveness of presynaptic 5-HT 1A receptor and an increase in the responsiveness of postsynaptic 5-HT 1A receptor occurs in adaptation to stress (Haleem, 1999; Haleem *et al*., 2002). When presynaptic receptors are desensitized their negative feedback action would become less effective. This would lead to a large increase in 5-HT concentration at the functional postsynaptic site. It has been suggested (Haleem, 1999; Haleem *et al*., 2002) that the presynaptic changes together with stress demand to produce adaptation to stress. The present study shows that administration of 8-OH-DPAT could attenuate stress-induced behavioral deficits but the time course of adaptation to stress essentially remain unaltered.

CONCLUSION

The present study was designed to examine the effects of prior administration of 8-H-DPAT (0.25 mg/kg) on restraint induced behavioral deficits and adaptation to repeated restraint stress in rats. The result shows that the drug administration could attenuate stress-induced behavioral deficits but the time course of adaptation to stress essentially remain unaltered. The present study, therefore, support the notion that a decrease in the availability of 5-HT in terminal region due to the stimulation of presynaptic 5-HT 1A receptors could attenuate anxiogenic-like effects of stress. On the other hand, a desensitization of somatodendritic 5-HT 1A receptors may help coping the stress demand to produce adaptation to stress.

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