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Effectiveness of somatodendritic and/or postsynaptic 5-HT-1A receptors following exposure to single restraint stress

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Abstract: Effects of a selected dose of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) were studied on somatodendritic and/or postsynaptic 5-hydroxytryptamine (5-HT; serotonin)-1A receptors responsiveness following exposure to single restraint stress. Rats were restrained for 2-h. 24-h after the termination of restraint period, 8-OH-DPAT at the doses of 0.25 mg/kg and saline (1ml/kg), was injected to unrestrained and restrained animals. Activity in a light dark box was monitored. Intensity of 8-OH-DPAT-induced serotonin syndrome was monitored for 5-30 min post injection. Rats were decapitated 1-h post-injection to collect brain samples for neurochemical estimation by high performance liquid chromatography with electrochemical detection (HPLC-EC). An episode of 2-h restraint stress decreased 24-h cumulative food intakes and changes in growth rates. Administration of 8-OH-DPAT increased time spent in light compartment in both unrestrained and restrained animals. Time spent in light compartment was smaller in 8-OH-DPAT injected restrained than unrestrained animals. Intensity of 8-OH-DPAT-induced serotonin syndrome monitored next day was smaller in restrained than unrestrained animals. Restrained animals injected with saline exhibited an increase in 5-HT and 5-hydroxyindolacetic acid (5-HIAA) levels in the hippocampus, hypothalamus, midbrain and cortex but not in the striatum. 8-OH-DPAT decreased 5-HT and 5-HIAA levels in different brain regions of unrestrained and restrained animals. The decreases were greater in restrained than unrestrained animals, suggesting a supersensitivity of somatodendritic 5-HT-1A receptors. Stimulation of somatodendritic 5-HT-1A receptor following exposure to an episode of 2-h restraint stress decreased the functional activity of postsynaptic 5-HT-1A dependent responses. 8-OH-DPAT decreased 5-HT and 5-HIAA levels more in restrained than unrestrained animals, suggesting an increase in the effectiveness of somatodendritc 5-HT-1A receptor responsiveness following exposure to single restraint stress.

Keywords: Restraint stress, 8-OH-DPAT, serotonin syndrome, somatodendritic, postsynaptic 5-HT-1A receptor.
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INTRODUCTION

Rats exposed to 2-h of restraint stress exhibited behavioral deficits[1-3]. Similar studies also showed that an episode of 2-h restraint stress decreased food intake and growth rate in rats[2]. On repeated immobilization of 2-h/day for 5 days, the behavioral deficits were no longer observed. It was suggested that repeated exposure to an uncontrollable stressor could induce adaptive changes that led to behavioral tolerance[4]. Conversely, failure to adapt to repeated stress is a depression model[1, 5]. Serotonergic response to stress is also a part of this adaptive mechanism. Thus, the whole brain and regional 5-hydroxytryptamine (serotonin; 5-HT) metabolism increased following exposure of rats to 2-h restraint, the increases did not occur in rats restrained 2-h/day for 5 days[2], suggesting that adaptation also occurs in serotonin response to stress along with behavioral adaptation. It was shown in subsequent studies that a decrease in the responsiveness of somatodendritic 5-HT-1A[4, 6] and terminal 5-HT-1A[6] receptors may help coping with stress demand to produce adaptation to stress.

The prototypical 5-HT-1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) exhibits anxiolytic[7-11] and antidepressant-like[11-16] properties in animal models. Acute administration of 8-OH-DPAT stimulates presynaptic 5-HT-1A receptor to decreases 5-HT synthesis and turnover[11,17] and postsynaptic receptors to produce hyperactivity syndrome[18,19].

The present study was designed to monitor the responsiveness of pre and postsynaptic 5-HT-1A receptors following exposure to single restraint stress.

All drugs that directly or indirectly increase central serotonin neurotransmission at postsynaptic 5-HT-1A and 5-HT-2A receptors can produce serotonin syndrome[20]. In the present study 8-OH-DPAT, a selective 5-HT-1A agonist was injected at a dose (0.25 mg/kg) that has been previously shown to elicit serotonin syndrome at a sub maximal intensity[18,21] The intensity of syndrome is compared in restrained and unrestrained animals. Effect of 8-OH-DPAT on the attenuation of brain serotonin metabolism, a somatodendritic response[17] is also monitored in unrestrained and restrained animals.

MATERIALS AND METHODS

Animals

Twenty-four locally bred male albino Wistar rats weighing 200–220gm purchased from The Agha Khan University, Pakistan were housed individually under a 12-h light and dark cycle (light on at 0600 h) with free access to cubes of standard rodent diet and tap water for 3 days before experimentation. All

experiments were performed according to a protocol approved by the local Animal Care Committee.

Drug

8-OH-DPAT HBr, purchased from Research Biochemicals (RBI, USA), was dissolved in saline at a dose of 0.25mg/kg and injected intraperitoneally (*ip*) in volumes of 1ml/kg. Control animals were injected with saline (1ml/kg).

Experimental protocol

Twenty-four animals randomly divided to two equal groups of 12 each were assigned as unrestrained and restrained. Animals of the restrained group were immobilized for 2-h commencing between 9:00 – 11:00 h. Animals of the unrestrained group was left to their home cage during this time.

Twenty-four-h after the termination of immobilization stress animals further divided to four groups of 6 each were assigned as (i) saline unrestrained; (ii) saline restrained; (iii) 8-OH-DPAT unrestrained; (iv) 8-OH-DPAT restrained were injected accordingly with saline (1ml/kg body weight) or 8-OH-DPAT (0.25mg/ml/kg body weight) at 9:00 - 10:00 h. Activity in a light-dark activity box was monitored at 9:30-10:30 h in saline and 8-OH-DPAT injected unrestrained and restrained animals. Intensity of serotonin syndrome elicited by 8-OH-DPAT was scored from 5 to 30 minutes post injection.

One-h after the drug and saline injections animals were sacrificed to collect brain samples hippocampus, hypothalamus, midbrain, cortex and striatum were removed which were stored at -70^{o}C for the estimation of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) by HPLC-EC.

Restraining procedure

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[2,13,22]. 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 animal rested on the Perspex plate.

Light-dark activity

The test was conducted in a locally-made compartment box [10]. The compartment of equal size (26x26x26 cm), with an access (12x12 cm) between the compartments, differed in their sensory properties. Walls of on compartment were light (transparent) and other dark (Black). A rat placed in this box expected to pass more time in the dark compartment. To determine the activity a rat was introduced via the dark compartment of the box. Time spent in the light compartment was monitored for a cut off time of 5 minutes.

8-OH-DPAT elicited 5-HT syndrome

Animals were placed individually in rectangular Perspex activity cages (26x26x26 cm) with sawdust covered floor 15 minutes before injecting 8-OH-DPAT. Forepaw treading and locomotion elicited by the drug were scored as described earlier by Haleem and Khan [18]. Unrestrained and restrained rats were placed in a separate observation cages and injected with 8-OH-DPAT were used in a balanced design. The number of cage crossings (movement in any direction with all four paws) and forepaw treading were scored for 1 min, every 5 min up to 30 min i.e. in 5 sessions of 1 min each. A total of 5 scoring periods was later determined.

Brain dissection technique

Animals were decapitated and the brain was removed immediately. Brain regions were dissected out as described earlier[2]. The cerebellum was pinched out by forceps. The brain dipped in ice cold saline was placed with dorsal side up in the molded cavity of a brain slicer. A fine fishing line wire was inserted into the slots of the slicer to make 1 mm thick slices of forebrain. Desired brain regions were identified with the aid of a stereotaxic atlas. Olfactory nucleus material was discarded. Punches of 2.5 mm diameter were made in the striatum and on two consecutive slices in the hypothalamus. Hippocampal material (CA 1-4 fields+subiculum+dentrate gyrus) was dissected out with a sharp scalpel. From remaining unsliced brain midbrain was dissected out with a scalpel cut made across the line of the brain stem.

HPLC-EC determination of 5-HT and 5-HIAA

Brain samples were homogenized as described previously [2]. 5-HT and 5-HIAA levels were determined by HPLC-EC as described before [21,23]. A 5µ Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used separation was achieved by mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu L-ECD-6A detector at an operating potential of 0.8 V. Calculations were done by an in line Shimadzu C-R6A Chromatopac.

Statistical analysis

Effects of restraint stress on 24 h cumulative food intake and growth rate were statistically analyzed by Student's T-test. Data on time spent in a light dark box in 8-OH-DPAT and saline injected unrestrained and restrained rats were analyzed by two-way ANOVA. Data on 8-OH-DPAT-induced hyperlocomotion and forepaw treading in unrestrained and restrained animals were also compared by T-test. Data on 8-OH-DPAT-induced

decreases of 5-HT metabolism in brain regions of unrestrained and restrained animals were analyzed by two-way ANOVA. Post-hoc analysis was done by Newman-Keuls test: *P* values < 0.05 were considered significant.

RESULTS

Figure 1 shows the effects of single (2 h) restraint stress on 24 h cumulative food intake and changes in growth rate. Data analyzed by Student's t-test showed food intake and growth rate were smaller in restrained than unrestrained animals.

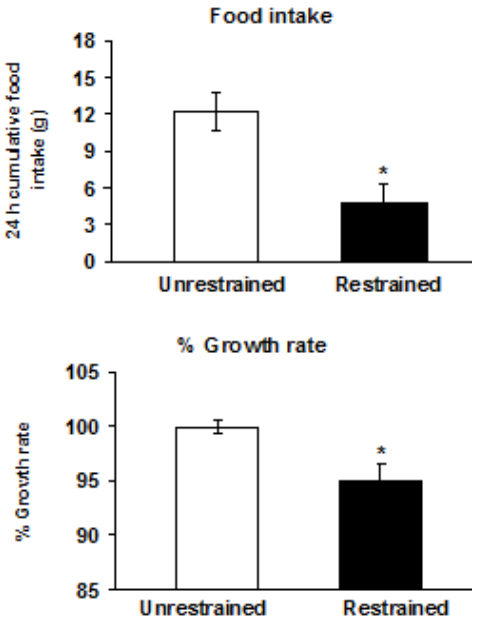

Figure 1: Effects of 2-h restraint stress on 24-h cumulative food intake and changes in growth rate. Values are mean±SD (n=12). *p<0.01 from unrestrained rats by t-test.

Figure 2 shows 8-OH-DPAT-induced hyperlocomotion, and forepaw treading in unrestrained and restrained animals. Data analyzed by Student's t-test showed that hyperlocomotion and forepaw treading were smaller in restrained than unrestrained animals.

Figure 3 shows the effects of single restraint on the activity of saline and 8-OH-DPAT injected animals in a light-dark box monitored as the time spent in light box. Two-way ANOVA performed on the data obtained 24-h after single restraint showed significant effects of 8-OH-DPAT administration ($F=30.4$ df=1,20 $p<0.01$) and stress ($F=54.6$ df=1,20 $p<0.01$). Interaction between the two factors ($F=0.07$ df=1,20 $p>0.05$) was not significant. Post-hoc analysis showed that an episode of 2-h restraint decreased time spent in the light compartment. Administration of 8-OH-DPAT increased time spent in the light compartment in unrestrained as well as restrained animals. 8-OH-DPAT injected restrained animals exhibited smaller values of time spent in light compartment than their unrestrained counterparts.

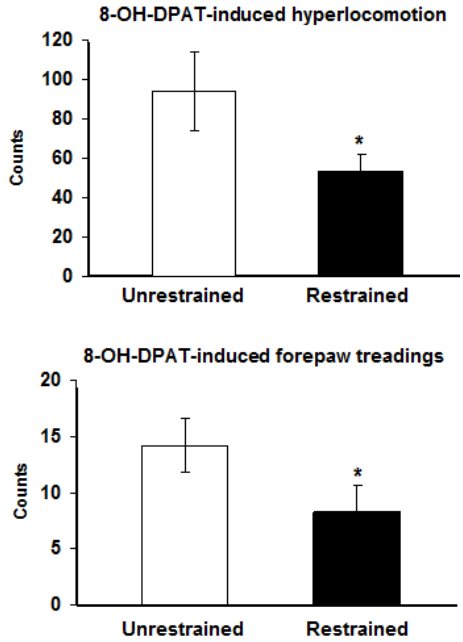

Figure 2: Component of 5-HT syndrome elicited by 8-OH-DPAT in unrestrained and restrained animals. Values are mean±SD (n=6) from 5-30 minutes post-injection and 24-h after the termination of restraint session. *p<0.01 from unrestrained rats by t-test.

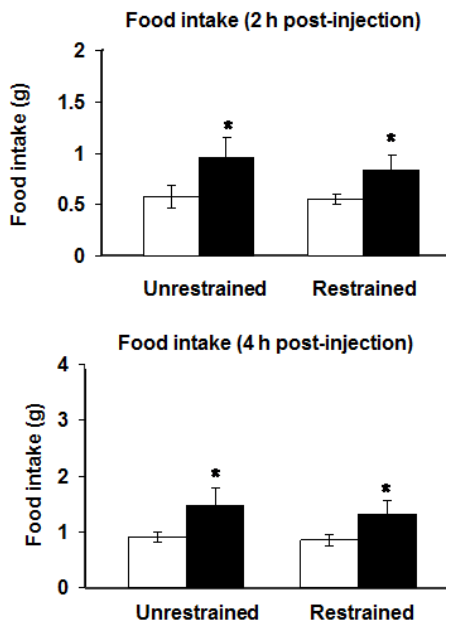

Figure 3: Light-dark activity in saline and 8-OH-DPAT injected unrestrained and restrained animals. Values areSD (n=6) 24-h after the single restraint stress. Significant differences by Newman-Keuls test: *p<0.01 from respective saline injected animals, +p<0.01 from respective unrestrained rats following Two-way ANOVA.

Figure 4 shows effects of administration of 8-OH-DPAT on 5-HT and 5-HIAA levels in different brain regions of unrestrained and restrained animals. Data on 5-HT levels analyzed by Two-way ANOVA (df=1,20) showed significant effects of 8-OH-DPAT for hippocampus ($F=134.32$ $p<0.01$), hypothalamus ($F=105.29$ $p<0.01$), cortex ($F=81.49$ $p<0.01$), midbrain ($F=68.52$ $p<0.01$) and striatum ($F=25.80$ $p<0.01$). Effects of stress were significant for hippocampus ($F=11.19$ $p<0.01$) and cortex ($F=17.44$ $p<0.01$) but not significant for hypothalamus ($F=2.32$ $p>0.05$), midbrain ($F=0.15$ $p>0.05$) and striatum ($F=0.004$ $p>0.05$). Interaction between stress and 8-OH-DPAT was significant for hippocampus ($F=41.30$ $p<0.01$), hypothalamus ($F=29.36$ $p<0.01$), cortex ($F=215.18$ $p<0.01$) and midbrain ($F=28.15$ $p<0.01$) but not significant for striatum ($F=2.42$ $p>0.05$). Post hoc analysis showed that an episode of 2-h restraint stress increased levels of 5-HT in the hippocampus, hypothalamus, cortex and midbrain but not in striatum. Administration of 8-OH-DPAT decreased 5-HT levels in the hippocampus, hypothalamus cortex, midbrain and striatum of unrestrained and restrained animals. 8-OH-DPAT-induced decreases of 5-HT were greater in the midbrain and hypothalamus but not in the hippocampus, cortex and striatum of restrained than unrestrained animals.

Data on 5-HIAA levels was analyzed by Two-way ANOVA (df=1,20) showed that the effects of 8-OH-DPAT were significant for hippocampus ($F=50.99$ $p<0.01$), hypothalamus ($F=96.94$ $p<0.01$), cortex ($F=52.85$ $p<0.01$), midbrain ($F=53.77$ $p<0.01$) and striatum ($F=105.38$ $p<0.01$). Effects of stress were significant for hippocampus ($F=9.41$ $p<0.01$), hypothalamus ($F=4.88$ $p<0.01$) and cortex ($F=18.99$ $p<0.01$) but not significant for midbrain ($F=2.77$ $p>0.05$) and striatum ($F=1.33$ $p>0.05$). Interaction between stress and 8-OH-DPAT were significant for hippocampus ($F=9.28$ $p<0.01$), hypothalamus ($F=19.47$ $p<0.01$), cortex ($F=23.43$ $p<0.01$), midbrain ($F=15.71$ $p<0.01$) and striatum ($F=9.26$ $p<0.01$). Post-hoc analysis showed that an episode of 2-h restraint stress increased levels of 5-HIAA in the hippocampus, hypothalamus, cortex and midbrain but not in the striatum.

Administration of 8-OH-DPAT decreased 5-HIAA levels in the hippocampus, hypothalamus, cortex, midbrain and striatum of unrestrained and restrained animals. 8-OH-DPAT-induced decreases of 5-HIAA levels were greater in the striatum but not in the hippocampus, hypothalamus, cortex and midbrain of restrained than unrestrained animals.

DISCUSSION

The behavioral responses produced by increasing the functional activity of serotonin following the administration of a monoamine oxidase inhibitor and l-tryptophan were first described by Hess and Doepfner[23] and subsequently by Grahame-Smith[24]. The most conspicuous signs of the so-called 5-HT behavioral syndrome are hyperactivity, reciprocal forepaw treading, head weaving and a flat body posture. The behavior also produced following the administration of agonists with selectivity towards 5-HT-1A receptors[1,18,21,25] is independent of pre synaptic machinery, as it was not blocked by the inhibition of 5-HT synthesis. A decrease in 8-OH-DPAT-induced locomotion by reserpine and haloperidol[9,26-28] suggests that dopamine D2 receptors are also involved in the consequences of this behavioral component of the syndrome.

Important finding of the present study is that an episode of 2-h restraint stress that produced behavioral deficits[1,3,29] comparable to an animal model of depression attenuates the intensity of 8-OH-DPAT-induced serotonin syndrome (Fig 2). The results provide evidence that an increase of brain serotonin metabolism and synthesis that occurs following exposure to an acute episode of 2-h restraint stress [2] is not able to increase serotonin function. Conversely, a decrease in the functional activity of serotonin is produced that may be involved in the precipitation of behavioral deficits observed in the animal model of depression.

Anxiolytic-like effects of restraint monitored in a light-dark activity box, were also attenuated by prior administration of 8-OH-DPAT (Figure 3) suggesting that the drug could attenuate anxiogenic effects of stress.

8-OH-DPAT, a full 5-HT-1A agonist activates somatodendritic as well as postsynaptic 5-HT-1A receptor to respectively decrease and increase serotonin neurotransmission[29-31]. The present study shows that 1h after the injection of 8-OH-DPAT at a dose of 0.25mg/kg, the decreases of 5-HT metabolism are significant in most brain regions of unrestrained and restrained animals. The present study shows that the stimulation of somatodendritic 5-HT-1A receptors decreases 5-HT and 5-HIAA concentrations more in restrained than unrestrained animals. It is therefore suggested that an increase in the effectiveness of somatodendritic 5-HT-1A receptors (Figure 4) leading to a decrease in the functional activity of 5-HT is involved in the precipitation of behavioral deficits observed in restrained animals. Conversely, a decrease in the effectiveness of somatodendritic 5-HT-1A receptors

that occurred following exposure to repeated restraint stress may help coping stress to produced adaptation to stress [6,17].

Pharmacological evidence shows that serotonin contributes to the suppression of eating behavior [32-34]. It is appearing from Fig. 1 and other studies that 2-h restraint stress decreased 24-h cumulative food intake and growth rate in rats[10,22,33]. Receptor research shows that increasing the functional activity of 5-HT at 5-HT-2C sites that are postsynaptic decreases food intake in experimental animals[15,35].

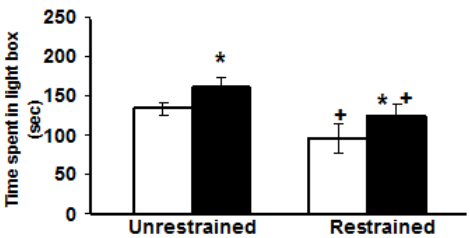

Figure 4: Effects of 8-OH-DPAT (0.25 mg/kg) on 5-HT and 5-HIAA levels in different brain regions of unrestrained and restrained animals. Values are mean±SD (n=6). 1-h after 8-OH-DPAT injection and 24-h after the termination of restraint periods. Significant differences by Newman-Keuls test: **p<0.01 and *p<0.05 from respective saline treated unrestrained and restrained animals, ++p<0.01 and +p<0.05 from respective saline treated unrestrained animals following two-way ANOVA.

In the present study effects of 8-OH-DPAT were monitored 24-h after the termination of restraint period. It is tempting to relate that decrease in the intensity of 8-OH-DPAT-induced serotonin syndrome (Figure 2) in restrained than unrestrained animals with an increase in the effectiveness of somatodendritic 5-HT-1A receptors (Figure 4).

In conclusion, the present study suggests that an episode of 2-h restraint stress sensitized somatodendritic 5-HT-1A receptors and decrease the responsiveness of postsynaptic 5-HT-1A receptors. It is suggested that an increase in the effectiveness of somatodendritic 5-HT-1A receptors may be atleast in part responsible for smaller 8-OH-DPAT-induced syndrome in restrained than unrestrained animals.

The results raise a possibility that functioning mediated via other 5-HT receptors such as 5-HT-2C receptors are also altered following exposure to restraint stress.

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