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Neurochemical and behavioral effects of 8-OH-DPAT following exposure to restraint stress in rats

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Abstract:
8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a selective 5-hydroxytryptamine 1A (serotonin; 5-HT_{1A}) agonist was used to evaluate the role of somatodendritic and/or postsynaptic 5-HT_{1A} receptors following exposure to restraint stress. Exposure to an episode of 2-h restraint stress decreased 24 h cumulative food intake. Intensity of 8-OH-DPAT-induced 5-HT syndrome monitored next day was smaller in restrained than unrestrained animals. Hyperphagic effects of 8-OH-DPAT were comparable in the two groups. Restrained animals injected with saline exhibited an increase in 5-HT levels in the hippocampus, hypothalamus and cortex but not in the midbrain and striatum. 5-Hydroxyindolacetic acid (5-HIAA) increased in the hippocampus, midbrain and cortex but not in the hypothalamus and striatum. 8-OH-DPAT injected at a dose of 0.25 mg/kg decreased 5-HT and 5-HIAA levels in different brain regions of unrestrained as well as restrained animals. The decreases were greater in restrained than unrestrained animals, suggesting a supersensitivity of somatodendritic 5-HT_{1A} receptors. The results are discussed in the context of a role of 5-HT_{1A} receptor in restraint-induced behavioral deficits.

Key words:
restraint stress, 8-OH-DPAT, 5-HT syndrome, hyperphagia, somatodendritic 5-HT_{1A} receptor

Introduction

The hypothesis that stress is the main predisposing and precipitating factor in the onset of depression is consistently supported by clinical observations [1, 2, 5, 11, 25]. Regarding the presentation of uncontrollable stress, a learned helplessness hypothesis was developed that assumed that rats previously exposed to an uncontrollable stress would subsequently show some behavioral deficits [30, 32, 37]. It is recognized that such rats perform worse in aversively motivated paradigms, are less active in an open field, and show the lack of pleasure-seeking behavior: for example, decreased response to attractive food and diminished performance of positively reinforced instrumental learning tasks [15].

In similar studies, an episode of 2-h restraint stress produced marked anorexia in rats [17, 19, 31, 33]. Exploratory activity monitored in an open field 24 h after the termination of stress also decreased. On repeated immobilization of 2 h/day for 5 days these behavioral deficits were no longer observed. It was suggested that repeated exposure to an uncontrollable stressor could produce adaptive changes that led to behavioral tolerance [17, 19, 26].

5-Hydroxytryptamine (5-HT; serotonin)-ergic responses to stress are also a part of this adaptive mechanism. Thus, rats exposed to an episode of 2-h restraint stress exhibited an increase in 5-HT metabolism and synthesis in many regions of the brain that did not occur after repeated immobilization of 2-h/day for 5 days [19] suggesting that adaptation in serotonin response to stress is also produced along with the behavioral adaptation.

A concept of receptor responsiveness in adaptation to stress emerged because the above results on the metabolism and synthesis of serotonin in adaptation to stress were not consistent with the 5-HT hypothesis of depression [7, 10, 15]. In view of 5-HT hypothesis of depression, an increase in 5-HT function would be expected to produce adaptation. It was, therefore, suggested that a change in receptor responsiveness is possibly involved in adaptation to stress [15]. In subsequent studies, we have shown that a desensitization of somatodendritic 5-HT_{1A} [16] and terminal 5-HT_{1B} [22] receptors is involved in adaptation to repeated restraint stress. It was suggested that when presynaptic receptors are desensitized, their negative feedback action would become less effective. The presynaptic change leading to a larger increase in 5-HT at the functional sites may be involved in adaptation to stress [22].

The present study is designed to monitor the responsiveness of somatodendritic and/or postsynaptic 5-HT_{1A} receptors following exposure to a single restraint period of 2 h. It was hypothesized that the responsiveness of somatodendritic 5-HT_{1A} receptors is increased following exposure to the stressor. A supersensitive negative feedback control on the synthesis and release of serotonin resulting in a decrease in the synaptic availability of 5-HT may precipitate behavioral deficits.

Materials and Methods

Animals

Twenty-four locally bred male albino Wistar rats weighing 200–220 g purchased from The Agha Khan University, Pakistan were housed individually under a 12-h light and dark cycle (light on at 06:00 h) with free access to cubes of standard rodent diet and tap water for 3 days before experimentation. All experiments were performed according to International European Ethical Standard and a protocol was 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 0.25 mg/kg and injected intraperitoneally (*ip*) in volumes of 1 ml/kg. Control animals were injected with saline (0.9%; 1 ml/kg).

Experimental protocol

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

Twenty four hours after the termination of immobilization stress animals were further divided into four groups of 6 rats each that were designated as (i) saline unrestrained; (ii) saline restrained; (iii) 8-OH-DPAT unrestrained; (iv) 8-OH-DPAT restrained, which were injected accordingly with saline (1 ml/kg) or 8-OH-DPAT (0.25 mg/ml/kg) at 9:00–10:00 h. Intensity of 5-HT syndrome elicited by 8-OH-DPAT was scored from 5 to 30 min post injection. Hyperphagic effects of 8-OH-DPAT were monitored as 2 h and 4 h food intake starting 30 min after 8-OH-DPAT or saline injection.

After this period animals were sacrificed to collect brain samples, hippocampus, hypothalamus, midbrain, cortex and striatum were removed and stored at –70°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" × 9" fitted with a Perspex plate of 9" × 6.5". Restraining procedure was the same as described earlier [19, 35]. 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.

8-OH-DPAT-elicited 5-HT syndrome

Animals were placed individually in rectangular Perspex activity cages (26 × 26 × 26 cm) with sawdust-covered floor 15 min 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 treadings 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 by Haleem and Perveen [20]. 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 + dentate 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 [20]. 5-HT and 5-HIAA levels were determined by HPLC-EC as described before [22, 35]. A 5 μ 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 Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu L-ECD-6A detector at an operating potential of 0.8 V.

Statistical analysis

Effects of restraint stress on 24-h cumulative food intake were statistically analyzed by Student's *t*-test. 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 hyperphagia and decrease in 5-HT and 5-HIAA levels 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 24-h cumulative food intake in unrestrained and restrained animals. Food intake of restrained animals 3.23 g/day was much smaller than the intakes of unrestrained animals (10.9 g/day). Data analyzed by Student's *t*-test showed a significant $p < 0.01$ decrease in food intakes following 2 h restraint stress.

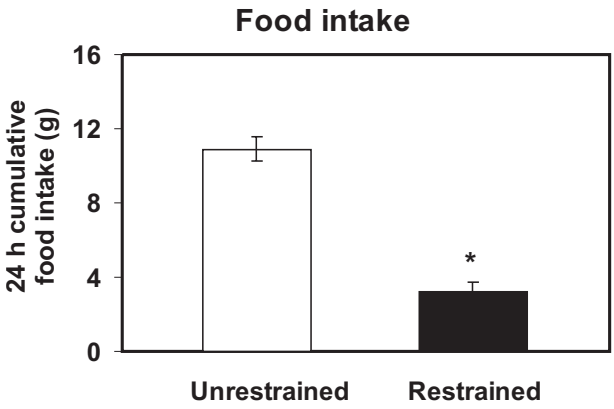

Fig. 1. Effects of 2-h restraint stress on 24-h cumulative food intake. Values are means ± SE (n = 12). * p < 0.01 *vs.* unrestrained rats by *t*-test

Figure 2 shows that administration of 8-OH-DPAT at a dose of 0.25 mg/kg elicited 5-HT syndrome in unrestrained as well as restrained animals. Data on the intensity of hyperlocomotion and forepaw treading analyzed by Student's *t*-test showed that the intensity

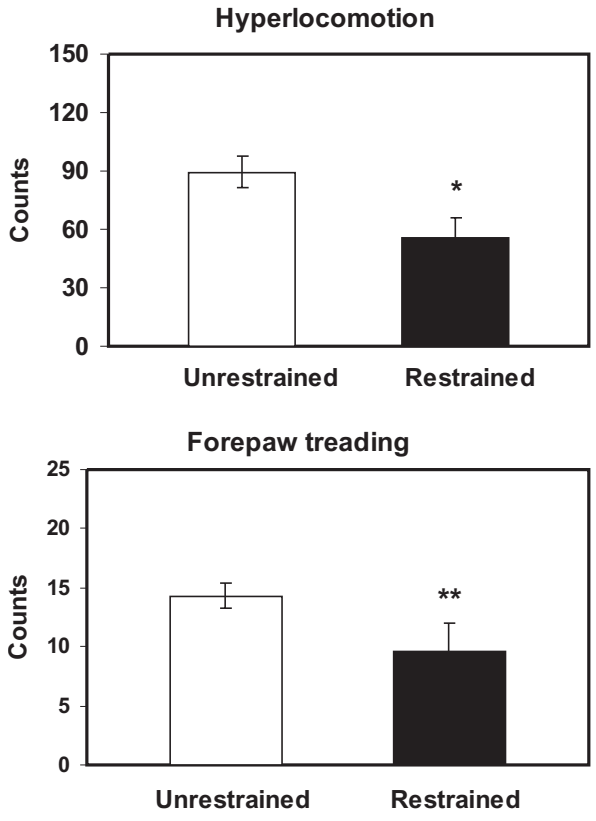

Fig. 2. Components of 5-HT syndrome elicited by 8-OH-DPAT in unrestrained and restrained animals. Values are the means ± SE (n = 6). Measurements were conducted from 5–30 min post-injection and 24 h after the termination of restraint session. * $p < 0.05$ and ** $p < 0.01$ *vs.* unrestrained rats (*t*-test)

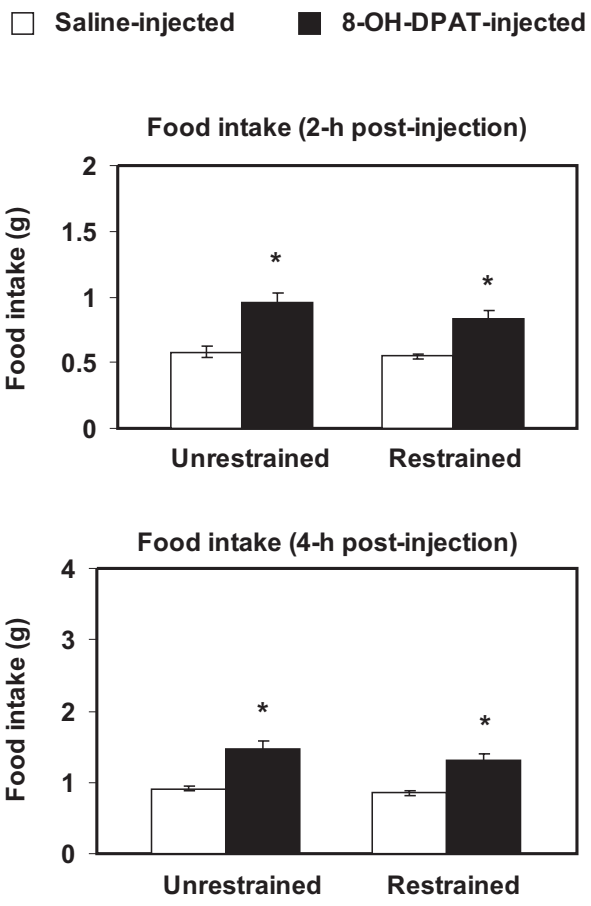

Fig. 3. Effects of 8-OH-DPAT (0.25 mg/kg) on 2 h and 4 h (post-injection) food intake in unrestrained and restrained animals. Values are the means ± SE (n = 6). Measurements were conducted 24 h after termination of stress and 2 h and 4 h after 8-OH-DPAT injection. Significant differences by Newman-Keuls test: * $p < 0.01$ *vs.* saline-treated unrestrained and restrained animals following two-way ANOVA

of both components was smaller in restrained than unrestrained animals.

Figure 3 shows that the administration of 8-OH-DPAT at a dose of 0.25 mg/kg at 2 h and 4 h elicited hyperphagia in unrestrained as well as restrained animals. Two-way ANOVA (df = 1, 20) showed significant drug effect at 2 h ($F = 34.18$ $p < 0.01$) and 4 h ($F = 32.13$ $p < 0.01$). Stress effects were not significant at 2 h ($F = 2.13$ $p > 0.05$) and 4 h ($F = 1.45$ $p > 0.05$). Interaction between stress and 8-OH-DPAT was not significant at 2 h ($F = 0.76$ $p > 0.05$) and 4 h ($F = 0.21$ $p > 0.05$) intakes. *Post-hoc* analysis showed that administration of 8-OH-DPAT increased food intake at 2 h and 4 h in unrestrained and restrained animals. The increases were comparable in the two groups.

Figure 4 shows the 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 drug effect for hippocampus ($F = 10.80$ $p < 0.01$), hypothalamus ($F = 4.66$ $p < 0.01$), midbrain ($F = 27.92$ $p < 0.01$), cortex ($F = 26.30$ $p < 0.01$) and striatum ($F = 15.24$ $p < 0.01$). Stress effects were significant for hippocampus ($F = 5.23$ $p < 0.05$), hypothalamus ($F = 20.56$ $p < 0.01$), midbrain ($F = 9.08$ $p < 0.01$), cortex ($F = 14.08$ $p < 0.01$) and striatum ($F = 5.10$ $p < 0.01$). Interaction between 8-OH-DPAT and stress was significant for hippocampus ($F = 10.10$ $p < 0.01$) but not for hypothalamus ($F = 1.94$ $p > 0.05$), midbrain ($F = 2.82$ $p > 0.05$), cortex ($F = 3.87$ $p > 0.05$) and striatum ($F = 2.14$ $p >$

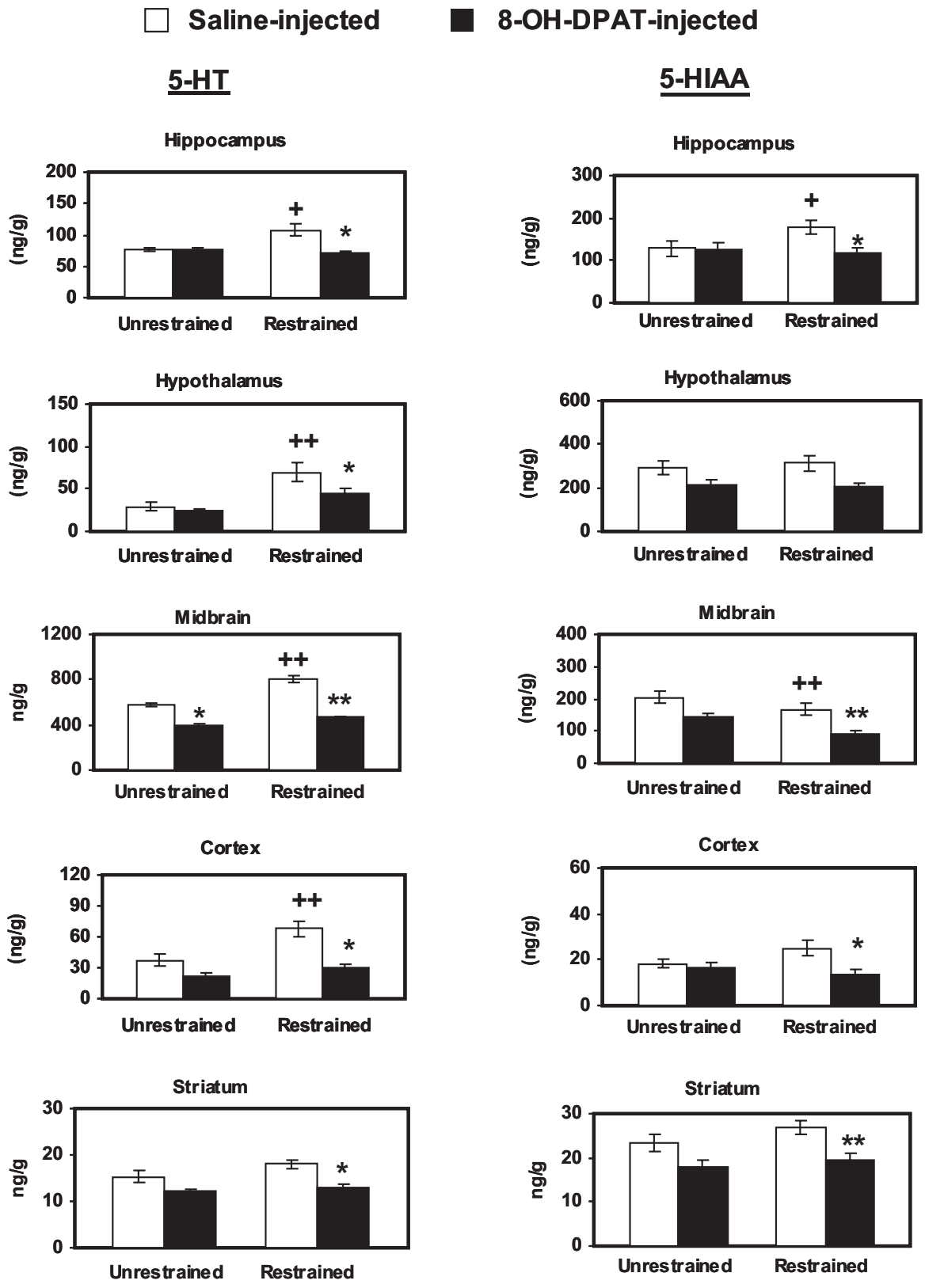

Fig. 4. Effects of 8-OH-DPAT (0.25 mg/kg) on 5-HT and 5-HIAA levels in different brain regions of unrestrained and restrained animals. Values are the means ± SE (n = 6). Measurements were conducted 4 h after 8-OH-DPAT injection and 24 h after the termination of restraint periods. Significant differences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

Data on 5-HIAA levels analyzed by two-way ANOVA (df = 1, 20) showed that effects of 8-OH-DPAT were significant for hippocampus ($F = 4.36$ $p < 0.05$), hypothalamus ($F = 10.88$ $p < 0.01$), midbrain ($F = 12.90$ $p < 0.01$), cortex ($F = 6.30$ $p < 0.05$) and striatum ($F = 15.33$ $p > 0.01$). Effects of stress were not significant for the hippocampus ($F = 2.10$ $p > 0.05$), hypothalamus ($F = 0.087$ $p > 0.05$), cortex ($F = 0.75$ $p > 0.05$) and striatum ($F = 2.03$ $p > 0.05$), but significant for the midbrain ($F = 8.44$ $p < 0.01$). Interaction between 8-OH-DPAT and stress was not significant for the hippocampus ($F = 3.96$ $p > 0.05$), hypothalamus ($F = 0.312$ $p > 0.05$), midbrain ($F = 2.10$ $p > 0.05$), cortex ($F = 3.21$ $p > 0.05$) and striatum ($F = 0.43$ $p > 0.05$). Post-hoc analysis showed that an episode of 2 h restraint stress increased levels of 5-HIAA in the hippocampus but not in the hypothalamus, midbrain, cortex and striatum. Administration of 8-OH-DPAT decreased 5-HIAA levels in the hippocampus, midbrain, cortex and striatum but not in the hypothalamus of restrained animals. 8-OH-DPAT-induced decreases of 5-HIAA were not significant in unrestrained animals.

Discussion

The behavioral responses produced by increasing the functional activity of 5-HT following the administration of a monoamine oxidase inhibitor and L-tryptophan were first described by Hess and Doepfner [23] and subsequently by Grahame-Smith [12]. 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 selective for 5-HT_{1A} receptors [13, 20, 21, 29, 37] is independent of presynaptic machinery, as it was not blocked by the inhibition of 5-HT synthesis. Hyperlocomotive responses to 8-OH-DPAT were also increased after the administration of neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) [11].

Important finding of the present study is that an episode of 2-h restraint stress attenuated the intensity of 8-OH-DPAT-induced 5-HT syndrome (Fig. 2). The results provide evidence that postsynaptic 5-HT_{1A} receptor-dependent functions are attenuated following exposure to an uncontrollable stressor and are involved in stress-induced behavioral deficits [19].

The availability of 5-HT at terminal/postsynaptic sites is under the control of an effective feedback mechanism. 5-HT_{1A} receptors located on the cell body and dendrites of serotonergic neurons and 5-HT_{1B} receptors located on the nerve terminal end are the mediators of the control mechanism. 8-OH-DPAT, a full 5-HT_{1A} agonist activates somatodendritic as well as postsynaptic 5-HT_{1A} receptor to decrease 5-HT synthesis and release in the terminal regions [4, 13, 18]. A decrease in whole brain and brain regional 5-HT metabolism 1 h after the administration of 8-OH-DPAT (0.25–1.0 mg/kg) has been shown in other studies [6, 34]. The present study shows that 4 h after the injection of 8-OH-DPAT at a dose of 0.25 mg/kg, the decreases in 5-HT metabolism are significant in most brain regions of restrained animals. The present study shows that the stimulation of somatodendritic 5-HT_{1A} receptor decreases 5-HT and 5-HIAA concentrations more in the terminal brain regions of restrained than unrestrained animals. It is, therefore, suggested that an increase in the effectiveness of somatodendritic 5-HT_{1A} receptors (Fig. 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 be involved in adaptation to stress [15].

Pharmacological evidence shows that 5-HT contributes to the suppression of eating behavior [2, 8, 14]. It is apparent from Figure 1 and other studies that 2-h restraint stress decreased 24-h cumulative food intake in rats [19, 32, 33, 36]. Research shows that increasing the stimulation of postsynaptic 5-HT_{2C} receptor decreases food intake [27, 28]. Conversely, the stimulation of somatodendritic 5-HT_{1A} receptors by selective agonists elicits hyperphagia [3, 24, 38] because the availability of 5-HT at functional postsynaptic hypophagic sites is decreased [3, 9, 24]. It has also been shown that activation of somatodendritic 5-HT_{1A} receptors by 8-OH-DPAT at a dose of 1 mg/kg attenuates restraint-induced anorexia for 3 h but not for 9 h [31]. Restraint-induced 5-HT release in the lateral hypothalamus also decreased [31].

In the present study, 8-OH-DPAT was injected 24 h after the termination of restraint period. Hyperphagic effects of 0.25 mg/kg 8-OH-DPAT that occur over a period of 2 h and 4 h did not occur over a period of

24 h in the present study (Fig. 3) and previous study [12]. It is tempting to relate the decrease in the intensity of 8-OH-DPAT-induced 5-HT syndrome (Fig. 2) in restrained *vs.* unrestrained animals with an increase in the effectiveness of somatodendritic 5-HT$_{1A}$ receptors (Fig. 4). On the other hand, comparable hyperphagic effects of 8-OH-DPAT in restrained and unrestrained animals cannot be explained on the same lines because a greater responsiveness of somatodendritic 5-HT$_{1A}$ receptors would be expected to elicit greater 8-OH-DPAT-induced hyperphagia in restrained than unrestrained animals. It is, however, possible that following exposure to restraint stress the responsiveness of hypophagic 5-HT$_{2C}$ receptors is also increased resulting in comparable 8-OH-DPAT-induced hyperphagia in restrained and unrestrained animals. It may be interesting to monitor the responsiveness of 5-HT$_{2C}$ receptors following exposure to restraint stress.

In conclusion, the present study shows that exposure to a stress-inducing situation increases the effectiveness of somatodendritic 5-HT$_{1A}$ receptors. The consequent decrease in the availability of 5-HT in terminal brain regions may precipitate behavioral deficits in the learned helplessness model. A greater decrease in the availability of 5-HT in terminal region such as hypothalamus would be expected to elicit greater hyperphagia in restrained than unrestrained animals. Comparable hyperphagic effects of 8-OH-DPAT in restrained and unrestrained animals as observed in the present study cannot be explained in terms of an increase in the effectiveness of somatodendritic 5-HT$_{1A}$ receptors. It is, however, possible that the effectiveness of postsynaptic hypophagic 5-HT$_{2C}$ receptors is also increased following exposure to restraint stress. In the present study, effects of 8-OH-DPAT on 5-HT and 5-HIAA concentrations were monitored 24 h after the termination of stress period and 4 h after the drug administration. Because behavioral deficits in the learned helplessness model were also monitored 24 h after the termination of stress period, in future studies, it may be important to monitor the effects of 8-OH-DPAT on brain regional 5-HT and 5-HIAA concentrations 1 h after the drug administration.

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