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## **Attenuation of Restraint-induced Anorexia and Anxiogenic Behavior by Serotonin-1A Agonists in Rats**

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The present study was designed to investigate the effects of buspirone (a partial 5-HT 1A receptor agonist) and 8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT, a full 5-HT 1A receptor agonist) on restraint-induced behavioral deficits. Exposure to a single episode of 2 h restraint stress decreased food intake, growth rate and elicited anxiogenic like behavior in rats. Prior administration of buspirone and 8-OH-DPAT attenuated stress-induced behavioral deficits of food intake and exploratory activity in a light-dark box. Novelty-induced anxiety in unrestrained animals was also diminished by prior administration of 8-OH-DPAT but not buspirone. The effects on the attenuation of restraint-induced anorexia were greater in 8-OH-DPAT than buspirone injected animals. The results are discussed in the context of a role of somatodendritic as well as postsynaptic 5-HT 1A receptors in the attenuation of restraint-induced behavioral deficits.

**Key words:** Stress, somatodendritic 5-HT 1A receptor, postsynaptic 5-HT 1A receptor, 8-OH-DPAT, buspirone

## INTRODUCTION

Rats exposed to 2 h restraint-stress exhibited decreased locomotion together with decreased food intake and growth rate<sup>[1,2]</sup>. On repeated immobilization these behavioral deficits were no longer observed<sup>[3,4]</sup>. A variety of stress stimuli increase the synthesis and turnover of 5-hydroxytryptamine (5-HT, serotonin) in whole brain and various brain region of rats<sup>[1,5-7]</sup>. When repeatedly restrained behaviorally adapted rats were challenged with an episode of 2 h restraint 5-HT synthesis did not increase in various brain regions<sup>[8]</sup>. In view of 5-HT hypothesis of depression, an increase in 5-HT function would be expected to produce adaptation to stress<sup>[8]</sup>. It was therefore argued that change in sensitivity of serotonin receptors is involved in adaptation to stress. Later study showed that following adaptation to stress the sensitivity of 5-HT 1A autoreceptors located on the cell soma and/or dendrites of serotonergic neurons is decreased<sup>[4]</sup> when these receptors are desensitized, their response would become less effective to increase synaptic availability of 5-HT<sup>[9]</sup>. Greater serotonin function may therefore lead to adaptation to stress.

Buspirone, a partial 5-HT 1A agonist<sup>[10]</sup> stimulates somatodendritic 5-HT 1A receptor and 8-OH-DPAT, a full 5-HT 1A agonist<sup>[11]</sup> acts via both somatodendritic as well as postsynaptic 5-HT 1A receptors. These drugs have been shown to elicit antianxiety<sup>[12]</sup> and antidepressant-like<sup>[13]</sup> effects in various animal models.

To get an insight in to the role of somatodendritic and/or postsynaptic 5-HT 1A receptors the present study concerns the effect of buspirone and 8-OH-DPAT on restraint-induced behavioral deficits in rats.

## MATERIALS AND METHODS

**Animals and treatment:** Thirty-six locally bred male albino Wistar rats weighing 230-275 g were housed individually in plastic cages with free access to cubes of standard rodent diet and tap water.

**Experimental protocol:** The animals divided into saline, buspirone and propranolol injected groups. Buspirone was injected at a dose of (0.2 mg kg<sup>-1</sup>) for 3 days<sup>[14]</sup>, while 8-OH-DPAT was injected at a dose of (0.25 mg kg<sup>-1</sup>) for 3 days<sup>[15]</sup>. Control animals were injected with saline in volume of (1 mL kg<sup>-1</sup>). Food intake and body weights were monitored during the treatment. After 3 days of drug administration animals of each group were further divided into unrestrained and restrained groups. Animals of restrained group were immobilized for 2 h (11:00 and 13:00 h). Animals of unrestrained group were left in their home cages during this time. Effects of restraint stress on

24 h cumulative food intake, body weight changes and exploratory activity in a light-dark box (for 5 min) were monitored next day.

**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<sup>[3]</sup>. 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 2 h of restraining period the animals were released and returned to their home cage.

**Light-dark activity test:** Exploratory activity in a light-dark box was monitored 24 h after the termination of 2 h restraint period. A rat placed in this box is expected to pass more time spent in the dark compartment. To determine the activity, a rat was placed in the light-dark compartment of the box. Time spent in the light compartment was monitored for a cut off time of 5 min.

**Statistical analysis:** Data were analyzed by Two-way ANOVA. Post-hoc analysis was done by Newman-Keuls test: p values < 0.05 were taken as significant.

## RESULTS

Two-way ANOVA performed on 24 h cumulative food intake in saline, buspirone and 8-OH-DPAT treated animals (Table 1) showed that effect of drug (F = 12.06, df = 2,30, p<0.01), stress (F = 144.51, df = 1,30, p<0.01) and interaction between stress and drugs (F = 5.64, df = 1,30, p<0.05) were significant. Post-hoc analysis by Newman-Keuls test showed that 2 h restraint decreased 24 h cumulative food intake in saline as well as buspirone and 8-OH-DPAT treated animals. The decreases were smaller in buspirone and 8-OH-DPAT treated than saline treated animals. 24 h cumulative food intakes were greater in 8-OH-DPAT than buspirone injected restrained animals.

Two-way ANOVA performed on 24 h changes of growth rate in saline, buspirone and 8-OH-DPAT treated animals (Table 2) showed significant effect of drugs (F = 6.95, df = 2,30, p<0.05), stress (F = 85.1, df = 1,30, p<0.01) and significant interaction between the two factors (F = 6.37, df = 1,30, p<0.05). Post-hoc analysis showed that an episode of 2 h restraint stress decreased growth rate in saline as well as in buspirone and 8-OH-DPAT injected animals. Growth rates of saline, buspirone and 8-OH-DPAT injected animals were largely comparable. 8-OH-DPAT injected unrestrained animals exhibited higher growth rate than saline and buspirone injected unrestrained animals.

Table 1: Effect of 2 h restraint stress on 24 h cumulative food intake in saline, buspirone and 8-OH-DPAT treated animals

Injections	Unrestrained	Restrained
Saline (1 mL kg <sup>-1</sup> )	14.4±3.7	2.2±1.8 <sup>*</sup>
Buspirone (0.2 mg kg <sup>-1</sup> )	15.8±2.5	5.7±1.1 <sup>*,+</sup>
8-OH-DPAT (0.25 mg kg <sup>-1</sup> )	16.7±2.4	8.4±2.5 <sup>*,**</sup>

Values are means±SD (n = 6). Significant differences by Newman-Keuls test, \*p<0.01 from respective unrestrained animals, <sup>\*</sup>p<0.05, <sup>\*\*</sup>p<0.01 from respective restrained rats following Two-way ANOVA

Table 2: Effect of 2 h restraint stress on 24 h changes of growth rate in saline, buspirone and 8-OH-DPAT treated animals

Injections	Unrestrained	Restrained
Saline (1 mL kg <sup>-1</sup> )	100.0±1.0	96.0±1.2 <sup>*</sup>
Buspirone (0.2 mg kg <sup>-1</sup> )	100.1±0.5	97.2±0.9 <sup>*</sup>
8-OH-DPAT (0.25 mg kg <sup>-1</sup> )	101.9±1.8 <sup>#</sup>	97.0±1.6 <sup>*</sup>

Values are means±SD (n = 6). Significant differences by Newman-Keuls test, <sup>\*</sup>p<0.01 from respective unrestrained, <sup>\*</sup>p<0.01 from saline injected unrestrained rats, <sup>#</sup>p<0.01 from buspirone treated unrestrained animals following Two-way ANOVA

Table 3: Effect of 2 h restraint stress on time spent in light dark compartment of a light dark box in rats treated with saline, buspirone and 8-OH-DPAT

Injections	Unrestrained	Restrained
Saline (1 mL kg <sup>-1</sup> )	80.0±9.8	52.3±7.2 <sup>*</sup>
Buspirone (0.2 mg kg <sup>-1</sup> )	88.8±16.3	78.6±15.0 <sup>*</sup>
8-OH-DPAT (0.25 mg kg <sup>-1</sup> )	160.8±12.6 <sup>#</sup>	124.8±9.02 <sup>*,#</sup>

Values are means±SD (n = 6) 24 h after the termination of stress. Significant differences by Newman-Keuls test, <sup>\*</sup>p<0.01 from respective unrestrained animals, <sup>\*</sup>p<0.01 from respective saline treated animals, <sup>#</sup>p<0.01 from respective buspirone injected animals following Two-way ANOVA

Two-way ANOVA performed on time spent in light box (light-dark activity) in saline, buspirone and 8-OH-DPAT treated animals (Table 3) showed significant effect of drugs (F = 120.21, df = 2.30, p<0.01), stress (F = 37.18, df = 1.30, p<0.01) and stress\*drug (F = 149.32, df = 1.30, p<0.01). Post-hoc analysis showed that an episode of 2 h restraint stress decreased time spent in light compartment in saline and 8-OH-DPAT treated animals but not in buspirone treated animals. Buspirone and 8-OH-DPAT injected restrained animals spent greater time in light compartment than saline treated restrained animals. Values in 8-OH-DPAT injected unrestrained as well as restrained animals were greater than the respective values in saline or buspirone treated animals.

## DISCUSSION

The effect of 2 h restraint stress on food intake (Table 1), growth rate (Table 2) and exploratory activity in a lit compartment (Table 3) largely agreed with previous data<sup>[3,16,17]</sup>. Shiamada *et al.*<sup>[18]</sup> reported that exposing rats to a stress inducing situation decreases exploration of light compartment in a light-dark activity box. In addition, the present study shows that administration of 8-OH-DPAT as well as buspirone attenuated restraint- induced deficits of lit box exploration. An increase in time spent in light compartment is used as a measure of anxiolytic profile of a drug<sup>[17,18]</sup>.

8-OH-DPAT and buspirone have been shown to produce anxiolytic effect in various models of anxiety<sup>[19,20]</sup>. There is substantial evidence for a key role of 5-HT 1A autoreceptors in mediating the anxiolytic effect of buspirone and 8-OH-DPAT<sup>[21]</sup>, in addition, several studies have provided a supporting role of postsynaptic 5-HT 1A receptors in the anxiolytic-like action of buspirone and other 5-HT 1A agonist<sup>[22,23]</sup>.

In the present study 8-OH-DPAT injected but not buspirone injected unrestrained animals also exhibited greater time spent in light box (Table 3). The results suggest that a full but not partial 5-HT 1A agonist could decrease novelty-induced anxiety.

Buspirone is a partial agonist at 5-HT 1A postsynaptic receptor, but a full agonist at 5-HT 1A presynaptic somatodendritic receptors<sup>[24]</sup>. It inhibits 5-HT cell firing<sup>[25]</sup> and thereby reduced 5-HT neurotransmission<sup>[26]</sup>. Acute administration of buspirone at doses from (1-20 mg kg<sup>-1</sup>) has been shown to decrease synthesis and release of 5-HT by stimulation of somatodendritic 5-HT 1A receptors<sup>[27]</sup>. It also reduced extracellular levels of 5-HT in the hippocampus<sup>[28]</sup>.

8-OH-DPAT a full agonist at 5-HT 1A receptors stimulate postsynaptic receptor to produce hyperactivity syndrome<sup>[29]</sup> and presynaptic receptors to decrease 5-HT turnover, synthesis and release by a feedback mechanism<sup>[30,31]</sup>. 8-OH-DPAT at a dose 60 µg kg<sup>-1</sup> sufficient to activate cell body but not postsynaptic 5-HT metabolism in the hippocampus<sup>[32]</sup>. Acute stimulation of somatodendritic 5-HT 1A receptor by 8-OH-DPAT decreased 5-HT metabolism and synthesis rate<sup>[33,34]</sup>. Sprouse and Aghajanian<sup>[25]</sup> have reported that activation of the somatodendritic 5-HT 1A receptor which are pre-dominantly located in the dorsal raphe nucleus result in an inhibition of the firing of 5-HT neuron. Consequently, leading to a decrease in 5-HT neurotransmission in their forebrain projection area<sup>[35]</sup>.

Regarding the anxiolytic effect after microinjection of 5-HT 1A agonist in brain region located postsynaptically to the serotonergic neurons. The stimulation of postsynaptic 5-HT 1A receptors might trigger a neuronal feedback loop terminating in the dorsal raphe nuclei, where it might exert an inhibitory influence on serotonergic neurons<sup>[36,37]</sup>.

The resultant decrease in 5-HT levels is thought to be involved in the anxiolytic effect of postsynaptic 5-HT 1A receptor stimulation.

The present study shows that stimulation of pre as well as postsynaptic 5-HT 1A receptors by buspirone and 8-OH-DPAT could attenuate restraint-induced behavioral deficits. Attenuation of novelty-induced anxiety by 8-OH-DPAT but not buspirone in unrestrained

animals is largely explicable in term of a feedback effect exerted on dorsal raphe neuronal firing by postsynaptic 5-HT 1A receptors. The finding may have implication in the treatment of stress-related disorders such as depression and anxiety.

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