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Anxiogenic and anorexiogenic effects of restraint stress in rats pretreated with buspirone or propranolol

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Abstract: The present study is designed to monitor the effects of buspirone and propranolol on responses to stress. Exposure to single (2-h) restraint stress decreased food intake, growth rate and time spent in the lit compartment of a light-dark activity box. The deficits were not observed following 5th (2-h/day) restraint stress, suggesting that adaptation occurred. Prior administration of buspirone and propranolol did not alter food intake and growth rates of unrestrained animals but attenuated restraint-induced deficits of food intake and growth rate. Administration of propranolol and buspirone resulted in an increase in time spent in light compartment of unrestrained animals and attenuated restraint-induced deficits of light-dark box activity, suggesting that the drugs could diminish novelty-induced as well as restraint-stress-induced anxiety. Adaptation in restraint-induced deficits of food intake, growth rate and anxiety was also produced in buspirone and propranolol treated animals after 5th restraint-stress of 2-h/day. The results are discussed in the context of a role of serotonin in the attenuation of restraint-induced behavioral deficits.

Keywords: Behavioral deficits, buspirone, propranolol, restraint stress, serotonin.
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INTRODUCTION

Stress is a feature of all lives. It acts as a predisposing and precipitating factor in the onset of affective illness specially depression[1]. Parallel studies on experimental animals show that an uncontrollable stressor produced neurochemical changes and behavioral deficits[2]. Similar studies also show that an episode of 2h restraint stress decreased food intake and growth rate in rats[3]. These behavioral deficits were no longer observed on repeated immobilization[4]. Single episode of 2h restraint increased 5-hydroxy tryptamine (5-HT; serotonin) synthesis in many brain regions of unrestrained rats[3,4]. These increases did not occur, when repeatedly restrained behaviorally adapted rats were restrained for 2 hours[3], suggesting that adaptation in serotonergic response to stress also developed together with the behavioral adaptation[3,4].

A role of 5-HT 1A receptors in adaptation to stress is of particular interest[4,5], because 5-HT 1A receptors are implicated in the pathogenesis of anxiety as well as depression[6]. The stimulation of presynaptic 5-HT 1A receptor in the median raphe nuclei elicits anxiolysis[7,8], whereas stimulation of postsynaptic 5-HT 1A receptor in its projection area is anxiogenic[7]. Studies have shown that adaptation to stress produces an increase in the responsiveness of postsynaptic 5-HT 1A receptor[3] and decrease in the sensitivity of presynaptic receptor[4].

The 5-HT 1A partial agonist buspirone[9-12] and antagonist propranolol[13-15] have been shown to have anxiolytic action in both animal and clinical studies.

Buspirone exerts its effects on serotonergic system[16-18] by activating 5-HT 1A autoreceptor[19,20]. It produces its anxiolytic activity by reduction of postsynaptic 5-HT mediated function[19-22] and reducing the density of 5-HT 2 receptor during long term administration[23,24]. Administration of buspirone has been shown to exhibit antidepressant-like activity[25-27]. It is often suggested that therapeutic effect of buspirone are not due to its immediate action on the 5-HT system, but rather adaptive changes that occur after prolonged treatment[10].

The activity of sympathetic nervous system could be antagonized by propranolol[28]. Propranolol is a 5-HT-1A/β-adrenoceptor antagonist[14-31] used to treat anxiety[32,33]. Zhang *et al*[34] reported that antidepressant-like effects of clenbuterol were antagonized by bilateral intra hippocampal infusion of propranolol. Pindolol and propranolol are reported to be useful as adjunctive medication in the treatment of depression by augmenting the antidepressant efficacy of specific serotonin reuptake inhibitor (SSRIs)[35].

The present study is designed to monitor the effect buspirone and propranolol on responses to stress in rats. The drugs were administered for two-weeks at doses that have been previously to elicit anxiolytic effects in animals[36-39].

MATERIALS AND METHODS

Animal and treatment

Thirty-six locally bred male albnio wistar rats weighing 230-275g purchased from The Aga Khan

University, Pakistan were housed individually under a 12-hours light and dark cycle (light on at 0600 hours) with free access of cubes of standard rodents diet and tap water for 3 days before experimentation. All experiments were performed according to a protocol approved by the Local Animal Care Committee.

Drugs

Buspirone and propranolol purchased from Sigma was dissolved in saline at a dose of 0.2 mg/kg and 1 mg/kg respectively and injected intraperitoneally (*i.p.*) in volume of 1 ml/kg body weight. Control animals injected with saline (1 ml/kg body weight).

Experimental protocol

The animals divided into saline (1 ml/kg), buspirone (0.2 mg/kg) and propranolol (1 mg/kg) injected groups received respective treatment once daily for 2 weeks.

After 2 weeks of drug administration, animals of each group were further divided into unrestrained and restrained groups. Animals of restrained groups were immobilized for 2-h (11:00 and 13:00 h) daily for 5 days. Animals of unrestrained groups were left in their home cages during this time. Effects of restraint stress on food intakes, body weight changes and activity in a light-dark box were monitored 24-h after the 1st and 5th restraint stress.

Behavioral methods

Restraint stress

The animals were restrained by immobilization stress. Restraining procedure was same as described earlier[3]. The animals were restrained by taping them on wire grids of 10″ x 9″ fitted with a Perspex plate of 9″ x 6.5″. Immobilization was effected 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. The animals were restrained between 11:00 to 13:00 h. After 2h of restraining period the animals were released and returned to their home cages.

Light-dark activity

The test was conducted in a locally-made 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 was expected to pass more time in the dark compartment. To determine the activity, a rat was introduced via the light compartment of the box. Time spent in the light compartment was monitored for a cut off time of 5 minutes.

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

Figure 1 shows the effect of single (2-h) and repeated (2-h/day for 5 days) restraint stress on 24h cumulative food intake in saline, buspirone and propranolol treated rats. Two-way ANOVA performed on single stress data showed significant effect of drugs ($F=18.04$ df=4,30 $p<0.01$), and stress ($F=169.53$ df=1,30 $p<0.01$). Interaction between stress and drugs was not significant ($F=2.70$ df=1,30 $p>0.05$). Post-hoc analysis by Newman-Keuls test showed that 2 h restraint stress decreased food intakes of saline as well as buspirone and propranolol treated animals. The decreases were smaller in buspirone and propranolol treated than saline treated animals.

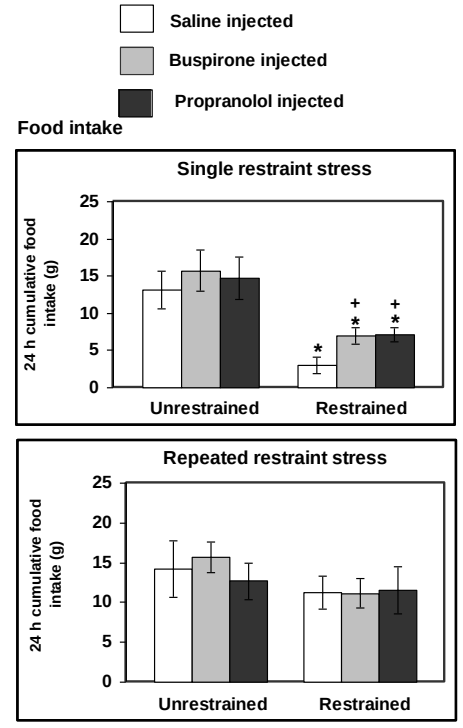

Figure 1: Effects of single and repeated (2-h/day for 5 days) restraint stresses on 24 h cumulative food intake in rats treated with saline, buspirone and propranolol. Values are means ± S.D. (n=6). Significant differences by Newman-Keuls test: *$p<0.01$ from respective unrestrained animals, +$p<0.01$ from respective saline treated animals following Two-way ANOVA.

Data on repeated (2-h/day for 5 days) restraint stress analyzed by Two-way ANOVA showed significant effect of stress (F=8.24 df=1,30 p<0.01). Effects of drugs were not significant (F=0.17 df=4,30 p>0.05). Interaction between stress and drugs was also not significant (F=1.23 df=1,30 p>0.05). Food intakes of unrestrained and restrained animals were highly comparable in all three groups.

Figure 2 shows the effect of single (2-h) and repeated (2-h/day for 5 days) restraint stress on growth rate in saline, buspirone and propranolol treated rats. Two-way ANOVA performed on single stress data showed significant effect of drugs (F=17.38 df=4,30 p<0.01), stress (F=176.95 df=1,30 p<0.01) and interaction between stress and drugs (F=12.46 df=1,30 p<0.01). Post-hoc analysis by Newman-Keuls test showed that 2-h restraint stress decreased growth rate in saline as well as buspirone and propranolol treated animals. The deficits of growth rate were smaller in buspirone and propranolol treated than saline treated rats.

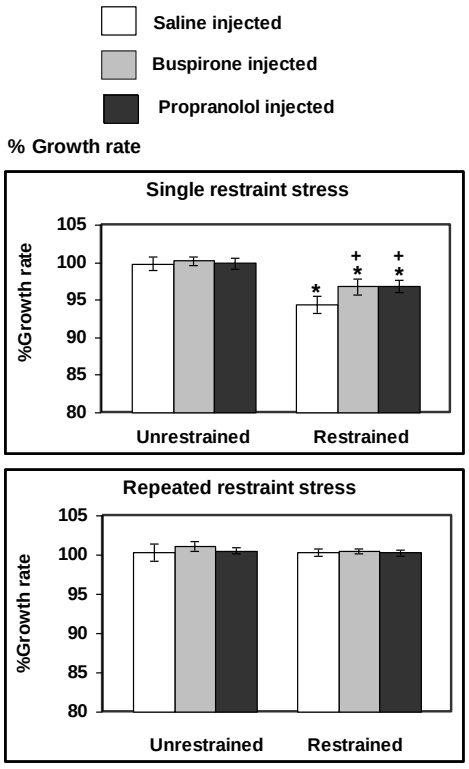

Figure 2: Effects of single and repeated (2-h/day for 5 days) restraint stresses on 24 h changes of growth rate in rats treated with saline, buspirone and propranolol. Values are means ± S.D. (n=6). Significant differences by Newman-Keuls test: *p<0.01 from respective unrestrained animals, +p<0.01 from respective saline treated animals following Two-way ANOVA.

Data on repeated (2-h/day for 5 days) restraint stress analyzed by Two-way ANOVA showed that the effects of drugs (F=1.50 df=4,30 p>0.05), stress (F=1.87 df=1,30 p>0.05) and interaction between stress*drugs (F=3.53 df=1,30 p>0.05) were not significant.

Figure 3 shows the effect of single (2-h) and repeated (2-h/day for 5 days) restraint stress on exploratory activity in light-dark activity box in rats treated with saline, buspirone and propranolol. Two way ANOVA performed on single stress data showed significant effect of drugs (F=10.54 df=4,30 p<0.01), stress (F=17.81 df=1,30 p<0.01) and interaction between stress and drugs (F=8.88 df=1,30 p<0.01).

The 2-h restraint stress decreased time spent in light compartment in saline, buspirone and propranolol treated animals, smaller in buspirone and propranolol than saline treated animals.

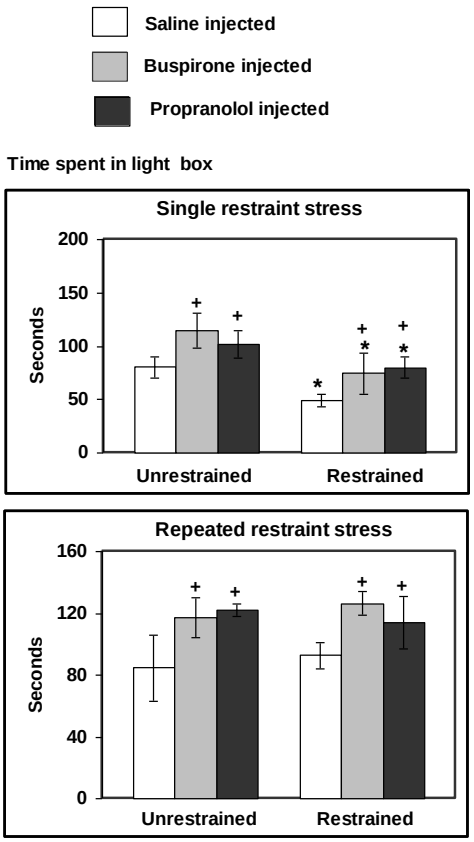

Figure 3: Effects of single and repeated (2-h/day for 5 days) restraint stresses on time spent in light compartment of a light-dark box in rats treated with saline, buspirone and propranolol. Values are means ± S.D. (n=6). Significant differences by Newman-Keuls test:*p<0.01from respective unrestrained animals, +p<0.01 from respective saline treated animals following Two-way ANOVA.

Data on repeated (2-h/day for 5 days) restraint stress analyzed by Two-way ANOVA showed that the effects of drugs ($F=0.21$ df=4,30 $p>0.05$), stress ($F=2.20$ df=1,30 $p>0.05$) and interaction between stress*drugs ($F=1.29$ df=1,30 $p>0.05$) were not significant. Post-hoc analysis by Newman-Keuls test showed that buspirone and propranolol treated unrestrained and restrained animals exhibited an increase in time spent in light compartment.

DISCUSSION

The effects of single (2-h) and repeated (2-h/day for 5 days) restraint stress on food intake and growth rate largely agreed with those of previous studies[3,40,41]. The present study together with the results of previous studies[3,40,41,] suggests that behavioral adaptation to stress schedule develops, when similar type of stress is administered repeatedly. In addition, the present study shows that prior administration of buspirone and propranolol for 2 weeks before exposing to restraint stress attenuated 2-h restraint-induced decreases of food intake and growth rate. Following repeated restraint stresses of 2-h/day for 5 days deficits of food intake and growth rate did not occur in saline or buspirone or propranolol injected animals (Figures 1 and 2).

Previously, it has been reported that rats exposed to 2-h restraint stress exhibit a decrease in exploration of light compartment in a light-dark activity box[42,-44]. In the present study also 2-h restraint stress decreased time spent in light compartment in saline treated animals. An increase in lit compartment exploration of buspirone and propranolol treated unrestrained animals is relevant with the anxiolytic profile of these drugs (Figure 3). The present study shows that both buspirone and propranolol could attenuate stress-induced deficits of exploratory activity. The results also show that the deficits of behavior are normalized in saline, buspirone and propranolol treated restrained animals, when the animals are exposed to the same stress repeatedly.

Previously, it has been shown that following adaptation to stress, the sensitivity of 5-HT 1A autoreceptor located on the cell soma and/or dendrites of serotonergic neurons is decreased, when these receptors are desensitized their negative feed back action on 5-HT metabolism and release would become less effective[4]. The resultant increase in 5-HT function at terminal region may therefore produce adaptation to stress[4].

Buspirone is a 5-HT autoreceptor agonist[12,45,46,47]. Acute administration of buspirone at doses 1-20 mg/kg has been shown to decrease the synthesis and release of 5-HT in terminal region [9]. Repeated administration of buspirone at a dose of 10 mg/kg for 2 weeks[27] and at a dose of 3 mg/kg for only 10 days[48] produced no effect on the synthesis of 5-HT. The normalization is considered to be due to the desensitization of somatodendritic 5-HT 1A autoreceptors[49]. The present results on the attenuation of restraint-induced behavioral deficits by buspirone are also explicable in term of a desensitization of somatodendritic 5-HT 1A receptors by the drug.

Propranolol, a ß-blocker, is a relatively weak antagonist of 5-HT[50]. The drug has moderate affinity for 5-HT 1A receptor[9]. Inhibitory effects of 5-HT on the firing of serotonergic neurons are not blocked by the administration of propranolol[50,51]. Effect of either 5-HT or 8-OH-DPAT on hippocampal neuronal firing activity are blocked by propranolol [52]. On the other hand 1-(2-methoxyphenyl)-4(4-(2-phtalimide)butyl)piperazine (NAN-190) and 8- (2- (4- (2 - methoxyphenyl) -1-piperazinyl) – 8 - azaspiro (4, 5) decane – 7 - dionedihydrochloride (BMY-7378) act as 5-HT 1A antagonists at postsynaptic sites have been shown to produce sub-maximal agonistic effects at presynaptic sites, inhibiting 5-HT neuronal firing. The suppression of raphe firing caused by NAN-190 and BMY-7378 was reinstated by propranolol[53,54]. Indeed, Fornal *et al.*[55] reported that systemic administration of propranolol produced a modest suppression of serotonergic neuronal activity in the dorsal raphe nucleus of freely moving cats. A reduction in stress-induced anxiety[28] following the administration of propranolol is often explained in terms of a decrease in stress-induced sympathetic stimulation[56]. An attenuation of restraint-induced behavioral deficits in rats treated with propranolol could also occur because of a decrease in restraint-induced sympathetic stimulation. It is also possible that the drug, an antagonist at somatodendritic 5-HT 1A receptor[57], decreases the effectiveness of negative feedback on 5- HT synthesis and release. A resultant increase in the synaptic availability of 5-HT may be involved in the attenuation of stress-induced behavioral deficits observed in propranolol injected animals.

In conclusion, the present study suggest that administration of buspirone and propranolol at doses and duration that have been previously shown to produce antidepressant effects in clinical studies[58-60] and animal research[61,62] could attenuate restraint-induced deficits of food intake and growth rate. In addition, both of these drugs attenuate novelty-

induced and stress-induced anxiety. Anxiolytic effects are explainable in term of agonist activity of buspirone towards somatodendritic 5-HT 1A receptors and a decrease in stress-induced sympathetic activity and/or antagonism of the effects of stress on somatodendritic 5-HT 1A receptors by propranolol.

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