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LACK OF RESTRAINT-INDUCED INCREASES OF BRAIN SEROTONIN METABOLISM IN RATS TREATED WITH SPIPERONE: RELATIONSHIP WITH RESTRAINT-INDUCED BEHAVIORAL DEFICITS

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ABSTRACT:

Spiperone is a potent dopamine (DA) D_2, serotonin (5-hydroxy tryptamine, 5-HT) 5-HT_{1A} and 5-HT_{2A} antagonist. It is used clinically as an antischizophrenic compound. Previous studies have shown that a downregulation of somatodendritic 5-HT_{1A} receptor is involved in adaptation to stress. The present study was designed to investigate the effects of spiperone administration on behavioral adaptation to an episode of restraint stress and on brain serotonin metabolism. Spiperone was administered to rats at a dose of 0.25 mg/kg/ml two times a day for two days. Saline or spiperone treated rats were restrained for 2h on day 2. Effects of restraint on food intake, water intake, growth rate, plus maze and open field activity were monitored on next day. All animals were killed after a restraint period of 2h on the 3rd day. An episode of 2h restraint decreased food intake, growth rate and water intake comparably in saline and spiperone treated rats. Open field activity was not altered by restraint stress or spiperone treatment. Plus maze activity decreased by restraint stress in saline but not spiperone treated rats. 5-HIAA levels increased in saline but not spiperone treated rats. The findings are discussed in the context of a role of serotonin and 5-HT_{1A} receptor antagonism in adaptation to stress.

INTRODUCTION

A number of stressors increase brain tryptophan and serotonin metabolism (Perez *et al.,* 1972). Deficiency of serotonin at important synaptic sites in the brain causes depression (Brown *et al.,* 1982). It has been reported that if a particular stress is repeated it may result in adaptation (Kennett *et al.,* 1985a). Adaptation to stress produces down regulation of presynaptic 5-HT_{1A} receptors (Haleem 1998, 1999; Haleem *et al.,* 2002). This would suggest that 5-HT_{1A} antagonists acting specifically at presynaptic sites may help in adaptation to stress. Spiperone is a widely used pharmacological tool that acts as a potent dopamine D_2, serotonin 5-HT_{1A} and serotonin 5-HT_{2A} antagonist (Metwally *et al.,* 1998). The drug has been shown to act as 5-HT_{1A} antagonist particularly at presynaptic sites (Blier *et al.,* 1993). The present study is designed to investigate the effects of spiperone on behavioral deficits responses to stress in rats. Effects on brain serotonin metabolism are also monitored.

MATERIALS AND METHODS

Locally bred Albino-Wistar rats weighing 200 to 250 grams were used for the experiment. They were caged individually in plastic cages with free access to cubes of standard rodent diet and

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tap water for 1-2 days before starting the experiment. In the beginning of experiment 24 animals were randomly divided into 12 saline treated (control) animals and 12 spiperone treated (test) animals. The saline and drug treated rats were again divided into restrained and unrestrained rats. The rats were given the standard rat diet and water throughout the experiment.

Drug Preparation:

Spiperone (Sigma) was suspended in minimum volume of saline and then drop-wise acetic acid added to dissolve the drug. Sodium hydroxide was added drop-wise and pH adjusted to 6.0. Required volume of saline was added and drug was injected at a dose of 0.25 mg/kg/ml.

Restraining Procedure:

The animals were restrained by an approved procedure as described before (Kennett *et al*., 1985b; Haleem and Parveen, 1994). The animals were restrained for 2h between 12:00-14:00 on specifically designed wire grids.

EXPERIMENTAL PROTOCOL

First Day Treatment:

Body weights, food intakes and water intakes of all rats were monitored. Freshly prepared spiperone (0.25 mg/kg/ml) and saline (0.9% NaCl) were injected to test and control rats respectively two times a day at 10:00 a.m. and 3:30 p.m.

Second Day Treatment:

Body weight, food intakes and water intakes of all rats were monitored. Saline and drug were injected to rats as on the 1st day. Animals (control and test groups) were divided into 6 unrestrained and 6 restrained rats). After 30 minutes of drug or saline injection animals of restrained group were immobilized on wire grids for 2 hours. After 2 hours restrained rats were released and returned to their home cages. Control and test animals were again injected with saline or spiperone respectively between 3:00-3:30 p.m.

Third Day Treatment:

Open field activity and open arm ambulatory activity on an elevated plus-maze were monitored between 9:00-11:00 a.m. Body weights, food intakes and water intakes were monitored and all the 24 rats were restrained for 2 hours. Immediately after the termination of restraint period all rats were killed by decapitation using guillotine. After decapitation of rats the brain were removed immediately and stored at -70°C until analysis.

BEHAVIORAL METHODS

Plus-maze test:

The plus-maze apparatus used in the present study consisted of four equal sized arms in which two were open and two were closed. Each arm was of 50 cm length and 10 cm width. Arms were joined by a central area of 5 cm^2. The height of the wall of closed arms was 40 cm. The maze was elevated from the floor at a height of 60 cm. To determine activity a rat was placed in the centre of the plus-maze and time spent in open and closed arm were monitored for 5 minutes. Number of entries of rats in open and closed arm during 5 minutes were also counted. The test is based on the principle that an animal placed on an elevated plus shaped maze passes vary little time in the open arm. This is because of the fear of the elevation of the maze and thinness of the open arm. For the type of apparatus used in the present study 5 minutes were thought to be sufficient for the animal

to explore the maze. Plus maze activities of control rats and drug treated rats were monitored in a balanced design to avoid order effect. Percentage of time spent in open area was calculated as:

$$\frac{\text{Time spent in open area (sec.)}}{\text{Total time (300 sec.)}} \times 100$$

Open field test:

The open field apparatus designed in our laboratory and made locally consisted of square area 76×76 cm with opaque walls 42 cm high. The floor was divided by lines into 25 equal squares. Testing was performed in a quiet room under white light as described by Kennett *et al*. (1985a) and Haleem *et al*. (1988). Animals taken out from their home cages were placed in the centre square of the open field (one at a time). Number of squares crossed with all four paws were counted for 5 minutes. Activities of control rats and drug (spiperone) treated rats were monitored in a balanced design to avoid order effect.

Neurochemical estimations:

Tryptophan, 5-HT, 5-HIAA in brain and tryptophan in plasma were made by HPLC-EC as described earlier (Haleem and Perveen, 1994).

Statistical Analysis:

Data on the effects of spiperone on food intake, growth rate and water intake were statistically analyzed by student's t-test. Neurochemical and behavioral data were analyzed by two-way ANOVA. Posthoc comparisons were made by Newman-Keuls test. P-value <0.05 were considered significant.

RESULTS

Effects of restraint stress on food intake, growth rate and water intake of saline and spiperone pretreated rats:

Fig. 1 shows the effect of 2h restraint stress on food intakes, water intakes and body weights in spiperone and saline treated rats. Data on food intake analyzed by 2-way ANOVA (df=1,20) showed non-significant effect of drug (F-Spiperone = 1.36, P>0.05), significant effect of stress (F-stress = 31.04, P<0.01) and non-significant interaction between two factors (F-interaction = 0.71, P>0.05). Posthoc analysis by Newman-Keuls test showed that restraint stress significantly decreased food intake in saline treated (P<0.01, 44%) as well as in spiperone treated (P<0.01, 38%) rats. Differences between saline and spiperone treated unrestrained animals were not significant. Saline and spiperone treated restrained animal also exhibited comparable values.

Data on growth rate analyzed by 2-way ANOVA (df=1,20) showed non-significant effect of drug (F-Spiperone=0.82, P>0.05), significant effect of stress (F-stress=25.83, P<0.01) and significant interaction between two factors (F-interaction=12.75, P<0.01). Posthoc analysis by Newman-Keuls test showed that restraint stress significantly decreased body weights of saline treated (P<0.01) but not spiperone treated (P>0.05) rats. Administration of spiperone also decreased body weights in unrestrained (P<0.01) rats but not in restrained (P>0.05) rats.

Data on water intake analyzed by 2-way ANOVA (df=1,20) showed a significant effect of drug (F-spiperone=12.26, P<0.01), non-significant effect of stress (F-stress=0.36, P>0.05) and non-significant interaction between two factors (F-interaction = 2.87, P>0.05). Posthoc analysis by Newman-Keuls test showed that restraint stress did not significantly decrease water intake in saline or spiperone treated rats. Administration of spiperone significantly decreased water intake in unrestrained (P<0.01) rats but not in restrained (P>0.05) rats.

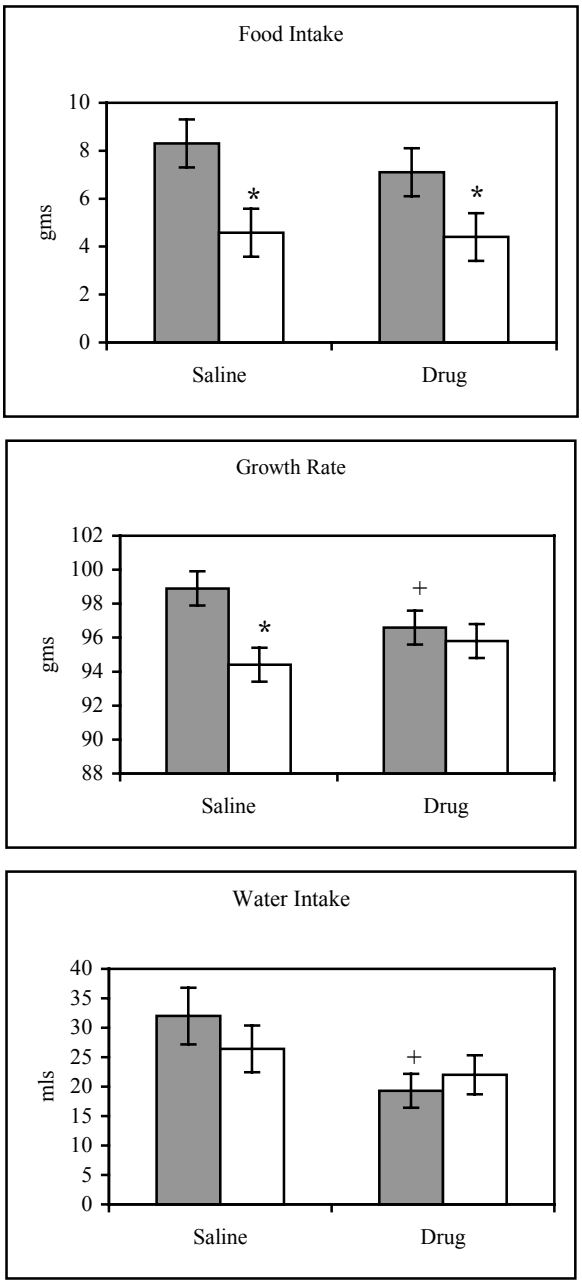

Fig.1: Effects of restraint stress on food intake, growth rate and water intake of saline and spiperone pretreated rats.
Values are means ± SD (n=6) of unstressed (filled column) and stressed (open column) rats. Significant differences by Newman-Keuls test. *$p<0.01$ from respective unrestrained rats, + $p<0.01$ from respective saline injected rats.

***Effects of 2h restraint on open field and plus maze activity in saline and spiperone pretreated rats*:**

Fig. 2 shows the effects of 2h restriant on open field activity in rats. Data for square crossed in an open field analyzed by two-way ANOVA (df=1,20) showed a non-significant effect of drug (F-spiperone = 0.18, >0.05), non-significant effect of stress (F-stress = 1.45, P>0.05) and non-significant interaction between two factors (F-interaction = 0.72, P>0.05).

Fig. 2 shows the effect of 2h restraint stress on plus maze activity in rats.

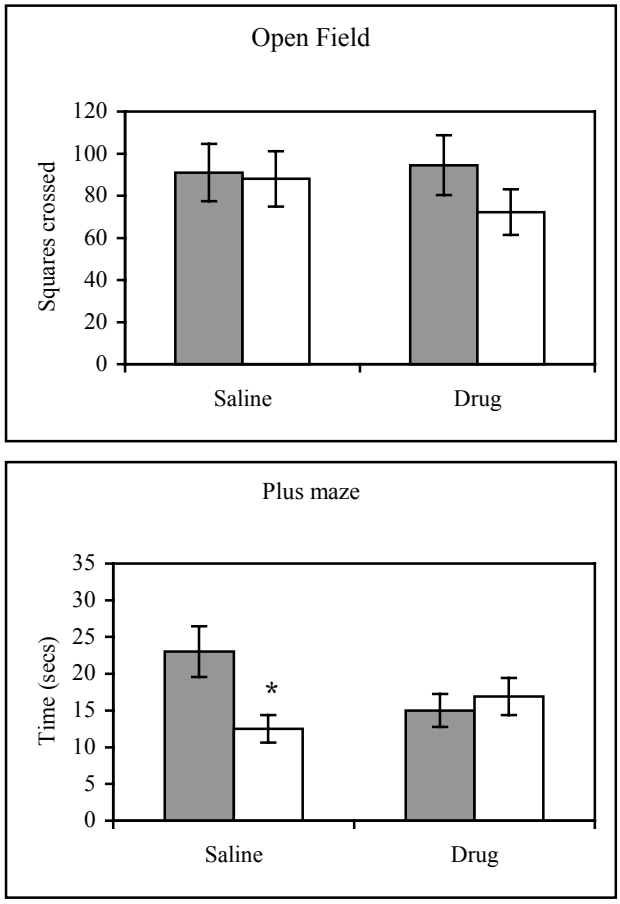

Fig.2: Effects of 2h restraint on open field and plus maze activity in saline and spiperone pretreated rats.
Values are means ± SD (n=6) of unstressed (filled column) and stressed (open column) rats. Significant differences by Newman-Keuls test. *p<0.01 from respective unrestrained rats.

Data on plus-maze activity (time spent in open arm) analyzed by two-way ANOVA (df=1,20) showed non-significant effect of drug (F-spiperone = 0.59, P>0.05), non-significant effect of stress (F-stress = 3.57, P>0.05) and significant interaction between two factors (F-interaction = 7.26, P<0.05). Posthoc analysis by Newman-Keuls test showed that restraint stress significantly decreased (P<0.05) the time spent in open arm in saline treated rats but not in spiperone treated rats. Mean values of time spent in open arm were smaller in spiperone

treated unrestrained than saline treated unrestrained rats. Differences by Newman-Keuls test were not significant.

Effects of 2h restraint stress on plasma tryptophan, brain tryptophan, 5-HT and 5-HIAA in saline and spiperone pretreated rats:

Fig. 3 shows the effect of 2h restraint stress on plasma tryptophan. Brain tryptophan, 5-HT and 5-HIAA in saline and spiperone pretreated rats. Data on plasma tryptophan analyzed by two-way ANOVA (df=1,20) showed a significant effect of drug (F-spiperone = 5.95, $P<0.05$), significant effect of stress (F-stress = 6.06, $P<0.05$) and non-significant interaction between two factors (F-interaction = 0.69, $P>0.05$). Posthoc analysis by Newman-Keuls test did not show any significant difference between groups. Data on brain tryptophan analyzed by two-way ANOVA (df=1,20) showed non-significant effect of drug (F-spiperone = 0.725, $P>0.05$), non-significant effect of stress (F-stress = 1.07, $P>0.05$) and significant interaction between two factors (F-interaction = 3.93, $P<0.05$). Posthoc analysis by Newman-Keuls test did not show any significant difference between groups.

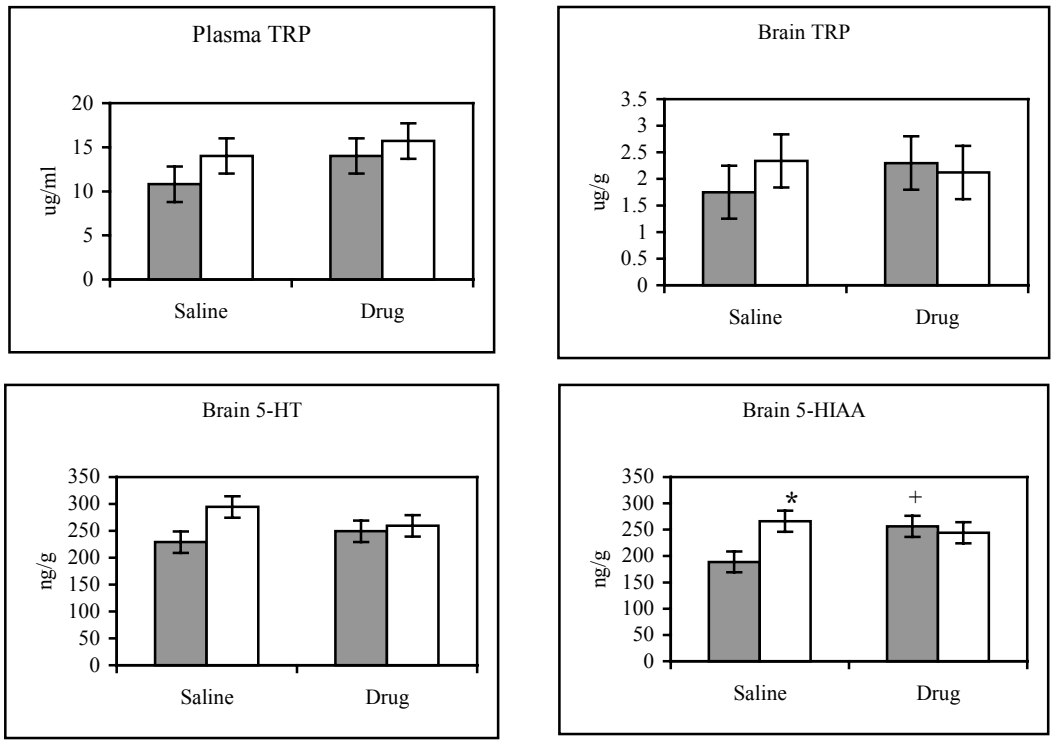

Fig.3: Effects of 2h restraint stress on plasma tryptophan, brain tryptophan, 5-HT and 5-HIAA in saline and spiperone pretreated rats.
Values are means ± SD (n=6) of unstressed (filled column) and stressed (open column) rats. Significant differences by Newman-Keuls test. *$p<0.01$ from respective unrestrained rats, + $p<0.01$ from respective saline injected rats.

Data on brain 5-HT analyzed by two-way ANOVA (df=1,20) showed a non-significant effect of drug (F-spiperone = 0.11, $P>0.05$), non-significant effect of stress (F-stress = 2.68, $P>0.05$) and non-significant interaction between two factors (F-interaction = 1.43, $P>0.05$).

Data on brain 5-HIAA analyzed by two-way ANOVA (df=1,20) showed non-significant effect of drug (F-spiperone=3.30, P>0.05), significant effect of stress (F-stress = 6.63, P<0.05 and significant interaction between two factors (F-interaction = 12.61, P<0.01). Posthoc analysis by Newman-Keuls test showed that restraint stress significantly increased brain 5-HIAA in saline treated (P<0.01) rats but not in spiperone treated (P>0.05) rats. Spiperone administration also increased 5-HIAA in unrestrained (P<0.01) rats but not in restraint rats.

DISCUSSION

Previously it has been shown that immobilization stress given for 2h decreased 24h cumulative food intake and growth rate. Locomotor activity monitored 24h later also decreased. The decrease of food intake, growth rate and locomotor activity were not observed when rats were restrained 2h/day for 5 days. It was suggested that adaptation to a stress schedule occurs when similar type of stress is administered repeatedly (Kennett *et al*., 1986; Kennett and Curzon, 1988; Haleem *et al*., 1988; Haleem and Perveen, 1994). There is considerable evidence supporting the role of serotonin in the control of food intake (Blundell, 1984). Previous studies also show that 2h restraint stress increased 5-HT synthesis rate in the hypothalamus and many other brain regions and decreased food intake and growth rate of previously unrestrained rats (Haleem and Parveen, 1994). In the present study, when saline treated rats were restrained food intakes and body weights were significantly decreased which is consistent with the previous studies (Kennett *et al*., 1986; Haleem and Perveen, 1994). Moreover, the present study shows that spiperone pretreatment did not attenuate restraint-induced deficit of food intake. Spiperone administration alone also did not alter food intake of unrestrained rats. An additional finding of the present study is that administration of spiperone decreased body weight and water intake in unrestrained rats. Although restraint stress did not decrease water intake in saline or spiperone treated rats. Body weights of both saline and spiperone treated rats decreased comparably by 2h restraint.

Previous studies show that rats exposed to 2h immobilization stress exhibited reduced locomotion in an open field 24h later (Kennett *et al*., 1985b). In the present study no effect of 2h restraint was observed on open field activity in saline or spiperone treated rats.

Arregui *et al*. (1993) have shown that administration of spiperone produces a general decline in active behavior. This decline in active behavior was not found in the present study when spiperone treated rats were tested in an open field. The results of plus-maze test however show that spiperone pretreatment produced slight decrease in the time spent in open arm. On the other hand, restraint stress decreased the time spent in open arm in saline treated but not in spiperone treated rats.

Acute stress increases brain serotonin turnover as brain levels of 5-HIAA increase but those of 5-HT are unaltered (Curzon *et al*., 1972; Morgen *et al*., 1975; Kennett and Joseph, 1981). The content of 5-HIAA the main catabolite of 5-HT, has been shown to be increased in the brain of the rats exposed to acute experimental stress (Adell *et al*., 1988; Dunn, 1988; Kennett and Joseph, 1981; Dunn and Welch, 1991). In the present study the concentration of 5-HT was not effected by stress or spiperone. However, 5-HIAA levels increased by both stress and spiperone. An increase in 5-HIAA by restraint stress has also been shown by other authors (Staudacher *et al.,* 1984 and Peters, 1990) and is consistant with studies reported from our laboratory (Haleem and Perveen, 1994). An increase in 5-HIAA concentration by spiperone is explicable in terms of 5-HT_{1A} antagonist activity of spiperone. Lack of restraint-induced increases of 5-HIAA in spiperone treated rats showed that blockade of somatodendritic 5-HT_{1A} receptors by spiperone could attenuate firing of serotonergic neurons.

In conclusion, the present results show that spiperone pretreatment attenuates behavioral deficits of restraint in a plus maze but the deficits of particularly food intake and body weight were not altered. Regarding the effects of stress on brain 5-HT metabolism and a role of presynaptic 5-HT_{1A} receptors in adaptation to stress, it is tempting to relate the attenuation of restraint-induced behavioral deficit on a plus maze with the lack of restraint-induced increase of 5-HIAA in rats treated with spiperone. These results strengthen suggestions previously made from our laboratory that absence of serotonergic response to stress is adaptive (Haleem and Perveen, 1994, Haleem *et al*., 2002).

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