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Behavioral and neurochemical profile of m-CPP following exposure to single restraint stress in rat

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Abstract

The present study was designed to monitor the responsiveness of 5-hydroxytryptamine (5-HT ; serotonin)-2C receptors following the exposure to restraint stress in rats. Rats were restrained for 2 hours. 24-h after the termination of the restraint period, 1-(m-Chlorophenyl)piperazine (m-CPP ; a 5-HT-2C agonist) at the doses of 1.5 mg/kg or 3.0 mg/kg and saline (1 ml/kg), was injected to unrestrained and restrained animals. Hypolocomotive effects (home cage activity, open field activity and motor coordination) and anxiogenic-like effects (light-dark activity and plus maze activity) were successively monitored in each animal after the drug or saline administration. Rats were decapitated 1-h post-injection to collect brain samples for neurochemical estimation by HPLC-EC. Our results show that differences in the hypolocomotive and anxiogenic-like effects of m-CPP between restrained and unrestrained animals could not be demonstrated in the present experimental paradigm. This was due to the fact that the behavioral effects were already marked after restraint leaving little room for an additional m-CPP effect. The effects of m-CPP on increasing 5-hydroxyindole acetic acid (5-HIAA) and decreasing dihydroxy phenyl acetic acid (DOPAC) levels were more pronounced ($p < 0.01$) in restrained than unrestrained animals. The results suggest that behavioral responses to m-CPP and stress are similar. The attenuation of m-CPP changes in 5-HIAA and DOPAC levels may be due to a decreased responsiveness of 5-HT-2C receptors in restrained animals.

Key words : Restraint stress ; 5-HT-2C receptor ; m-CPP ; Hypolocomotion ; Anxiogenic-like effect.

Introduction

The hypothesis that, stress is the major precipitating factor in the onset of depression is consistently supported by clinical observations (Anisman & Zacharko, 1982 ; Breslau & Davis, 1986 ; Gilbert *et al.*, 2004 ; Kendler *et al.*, 2004). Clinical findings stimulated experimental psychopharmacologists to create animal models of depression in which stressful stimuli have been used to produce learned helplessness or depressive state in animals. These models include procedures involving the presentation of uncontrollable stressors such as inescapable shock, restraint stress and other unpleasant treatments to the animals.

Parallel studies on experimental animals show that an uncontrollable stressor produces neurochemical and behavioral deficits. Failure to adapt following an uncontrollable stress is a model of depression (Weiss *et al.*, 1981). Episode of stress increases brain 5-hydroxytryptamine (5-HT ; serotonin) metabolism and synthesis rate in animal produces behavioral deficits comparable to an animal model of depression (Haleem & Perveen, 1994). In similar studies it has been reported that rats exposed to an episode of 2-h restraint stress exhibited a decrease in 24-h cumulative food intake and growth rate (Haleem & Perveen, 1994 ; Kennett *et al.*, 1986). Exploratory activity monitored in an open field 24-h after the termination of stress also decreased (Kennett *et al.*, 1986).

5-HT has been implicated in the etiology of many disease states and may be particularly important in mental illness such as anxiety and depression. During the last decade multiple receptors for 5-HT have been characterized. 5-HT-1A receptors are present on the soma and dendrites of 5-HT neurons and also on postsynaptic neurons in various brain regions of the limbic system (Verge *et al.*, 1985). Stimulation of somatodendritic receptors inhibits electrical activity in serotonergic neurons (Blier & De Montigny, 1987). Synthesis and release of 5-HT from nerve ending is also decreased (Haleem *et al.*, 1990 ; Adell *et al.*, 1991 ; Blier & Ward, 2003).

A role of 5-HT-2C receptor is known to occur in anxiety. Animal studies have demonstrated that stimulation of 5-HT-2C receptor is involved in anxiogenic-like activity (Alex *et al.*, 2005 ; Cornelio & Nuned-de-Souza, 2007). Drugs that tend to increase 5-HT functions are anxiogenic while blockade of serotonin neurotransmission produced antianxiety effect.

1-(m-Chlrophenyl)piperazine (m-CPP) is the major metabolite of the antidepressant drug, trazodone. Bauman *et al.* (1993 ; 2001) suggested that m-CPP may be a selective 5-HT-2C agonist, although it might have some 5-HT releasing properties. In-vitro binding studies showed that m-CPP binds to 5-HT-1A, 5-HT-1B/1D, 5-HT-2C and 5-HT-3 receptors in rat brain (Hoyer, 1988 ; Hamik and Peroutka, 1989 ; Fiorella *et al.*, 1995) and of these, binding was most potent at 5-HT-2C receptors. m-CPP increases 5-HT release (Hikiji *et al.*, 2004) via the stimulation of 5-HT-2C receptors (Gibson *et al.*, 1996) and decreases DA release in the striatum (Alex *et al.*, 2004) and other regions (Prisco *et al.*, 1996) of rat brain. The drug produces hypolocomtion (Lucki *et al.*, 1989 ; Gleason & Shannon, 1998), hypophagia (Harada *et al.*, 2006 ; Dalton *et al.*, 2006) and anxiogenesis (Kennett, 1992 ; Fone *et al.*, 1996) in rats.

The present study was designed to monitor the responsiveness of 5-HT-2C receptors following exposure to restraint stress.

Methods and material

Animals

Twenty four locally bred male albino Wistar rats weighing 180-220 g purchased from HEJ Research Institute, University of Karachi, Pakistan were housed individually with free access to cubes of standard rodent diet and tap water 3 days before starting the experiment.

Drugs

m-CPP purchased from Sigma was dissolved at a dose of 1.5 mg/kg or 3.0 mg/kg body weight. Control animals were injected with saline in volume of 1 ml/kg body weight.

Experimental protocol

Twenty-four animals randomly divided into 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 were left to their home cage during this time.

24-h after the termination of immobilization stress animals further divided to six groups of four each were assigned as (i) saline unrestrained (1 ml/kg) ; (ii) saline restrained (1 ml/kg) ; (iii) m-CPP unrestrained (1.5 mg/kg) ; (iv) m-CPP restrained (1.5 mg/kg) ; (v) m-CPP unrestrained (3.0 mg/kg) ; (vi) m-CPP restrained (3.0 mg/kg). Activity in a home cage, in an open field, motor coordination, time spent in the light compartment of a light-dark activity box and time spent in open arm of plus maze respectively were monitored 15 min, 25 min, 30 min, 35 min and 40 min of post-injections.

Animals were sacrificed 1-h after m-CPP or saline injection to collect the whole brain samples. Samples were stored at –70°C for the determination of brain indoleamine and catecholeamine.

Behavioral analysis

Restraining procedure

The animals were restrained on wire grids of $10^2 \times 9^2$ fitted with a Perspex plate of $9^2 \times 6.5^2$. Restraining procedure was same as described earlier (Haleem & Parveen, 1994). 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.

Home cage activity

To monitor activity in a familiar environment, activity boxes were used. The rectangular Perspex activity cage consisted of small square area ($26 \times 26 \times 26$ cm) with sawdust-covered floor. Before monitoring the activity an animal was placed in it for 15 minutes for habituation. Numbers of crossings across the box were monitored for 10 min.

Open field activity

To monitor activity in a novel environment, open field apparatus was used. The open field apparatus used in the present investigation consisted of a square area 76×76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity a rat was placed in the center square of the open field. The numbers of squares crossed with all four paws were scored for 5 min.

Motor coordination

Motor coordination was assessed on a Rota–rod (UGO-BASILE, Italy). The Rota-rod (Knurled

Perspex) with a drum of 7 cm radius and a speed of 2-20 revolutions/ min during training session and a fixed speed of 20 revolutions/ min during test session. The surface of the drum is not too glossy and smooth. A day before the treatment rats were trained in a single session until they attained 150 sec on Rota-rod. The latency to fall in a test session of 150 sec was taken as measure of motor coordination.

Light-dark activity

The test was conducted in a locally made compartment box. The compartment of equal size ($26 \times 26 \times 26$ cm), with an access (12×12 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 is expected to pass more time in the light 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 min.

Plus maze activity

The plus maze apparatus used in the present investigation was specially designed in our laboratory and it consisted of four arms in which two were open and two were closed. The arms were of identical length (50 cm) and width (10 cm). Arms were joined by central area of 5 cm^2. The maze was elevated from the floor at a height of 60 cm. To determine the activity a rat was placed in the center of the plus maze and time spent in the open arms were determined for 5 min.

Neurochemical analysis

HPLC-EC determination of indoleamine and catecholamine

Brain samples were extracted as described before (Haleem & Khan, 2003). A 5 mm ODS (Shim-Pack) separation column (4.5mm internal diameter and 15 cm length) was used. The mobile phase comprising methanol (14%), octyl sodium sulfate (0.02%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9, was passed at an operating pressure of 2000-3000 psi with the help of Schimadzu LC-6A pump. Electrochemical detection was achieved on Schimadzu LECD-6A detector at an operating potential of 0.8 volts.

Statistical analysis

Data on the effect of m-CPP administration on behavioral and neurochemical analysis of unrestrained and restrained animals analyzed by two-way ANOVA. Post-hoc comparison done by Newman-Keuls test : P values < 0.05 taken as significant.

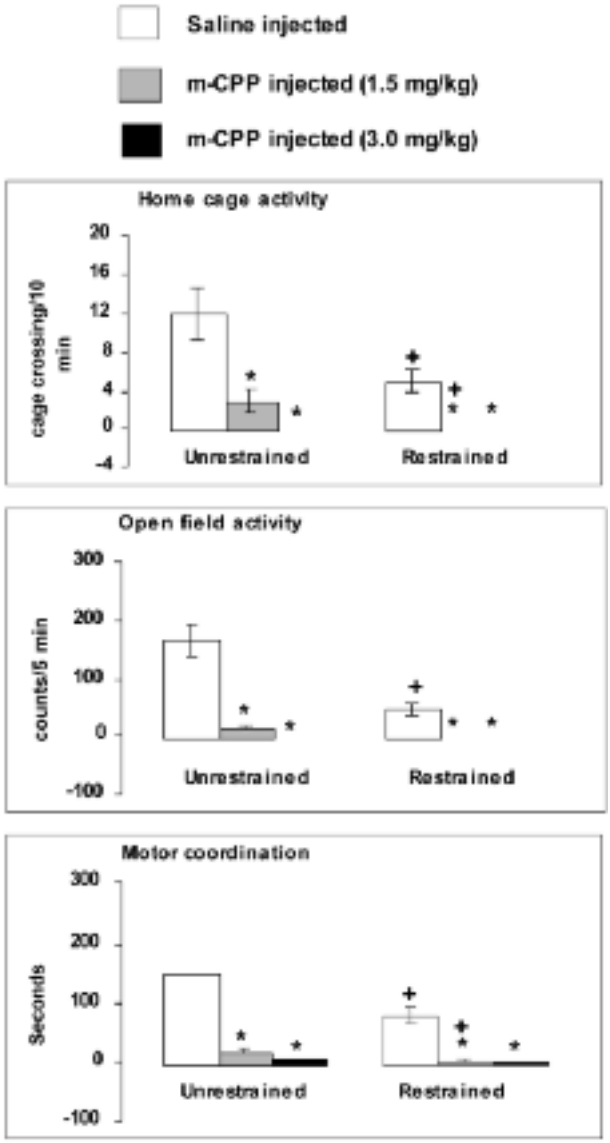

FIG. 1. — Effects of m-CPP administration on exploratory activity in home cage, open field and on motor coordination in unrestrained and restrained animals. Values are means ± S.D. (n = 4). Significant differences by Newman-Keuls test : *P < 0.01 from saline injected animals, +P < 0.01 from respective unrestrained animals following two-way ANOVA.

Results

Figure 1 shows the effects of m-CPP on exploratory activity in home cage, open field and on motor coordination. Data analyzed by two-way

ANOVA showed that effects of stress were significant on the activity in home cage ($F = 37.50$ $df = 1,18$ $p < 0.01$), open field ($F = 83.05$ $df = 1,18$ $p < 0.01$) and motor coordination ($F = 206.36$ $df = 1,18$ $p < 0.01$). Effects of m-CPP were also significant on the activity in home cage ($F = 180.18$ $df = 2,18$ $p < 0.01$), open field ($F = 410.75$ $df = 2,18$ $p < 0.01$) and motor coordination ($F = 2277.15$ $df = 2,18$ $p < 0.01$). Interaction between stress and m-CPP was significant for the activity in home cage ($F = 32.81$ $df = 1,18$ $p < 0.01$), open field ($F = 117.21$ $df = 1,18$ $p < 0.01$) and for motor coordination ($F = 200.86$ $df = 1,18$ $p < 0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of m-CPP at a dose of 1.5 mg/kg or 3.0 mg/kg decreased activity of unrestrained and restrained animals. Saline injected restrained animals exhibited impaired motor coordination and a decrease in activity in home cage and open field.

Figure 2 shows the anxiogenic-like effects of m-CPP in light-dark activity box and on elevated plus maze in unrestrained and restrained animals. Data analyzed by two-way ANOVA showed significant effect of stress on time spent in light box ($F = 112.23$ $df = 1,20$ $p < 0.01$) and in open arm ($F = 119.88$ $df = 1,18$ $p < 0.01$). Effects of m-CPP were significant on time spent in light box ($F = 340.76$ $df = 2,18$ $p < 0.01$) and open arm ($F = 239.77$ $df = 2,18$ $p < 0.01$). Interaction between stress and m-CPP was significant for time spent in light box ($F = 132.44$ $df = 1,18$ $p < 0.01$) and in open arm ($F = 95.28$ $df = 1,18$ $p < 0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of m-CPP at doses 1.5 mg/kg or 3.0 mg/kg decreased time spent in light box and open arm of unrestrained and restrained animals. Saline injected animals exposed to restraint stress also exhibited a decrease in time spent in light box and open arm, suggesting that both m-CPP and stress elicited anxiogenic-like effects in light-dark box and elevated plus maze.

Figure 3 shows the effect of m-CPP on 5-HT and 5-HIAA levels in unrestrained and restrained animals. Data analyzed by two-way ANOVA showed significant effects of stress on 5-HT ($F = 3.78$ $df = 1,18$ $p < 0.05$) and 5-HIAA ($F = 40.31$ $df = 1,18$ $p < 0.01$). Effect of m-CPP were also significant for 5-HT levels ($F = 9.42$ $df = 1,18$ $p < 0.01$) and 5-HIAA ($F = 60.96$ $df = 1,18$ $p < 0.01$). Interaction between stress and m-CPP were significant for 5-HT ($F = 6.59$ $df = 1,18$ $p < 0.01$) and 5-HIAA ($F = 14.80$ $df = 1,18$ $p < 0.01$). Post-hoc analysis by Newman-Keuls test showed that administration of m-CPP at a dose of 3.0 mg/kg increased 5-HT levels in unrestrained but not restrained animals. The levels of 5-HIAA increased at dose of 1.5 mg/kg or 3.0 mg/kg

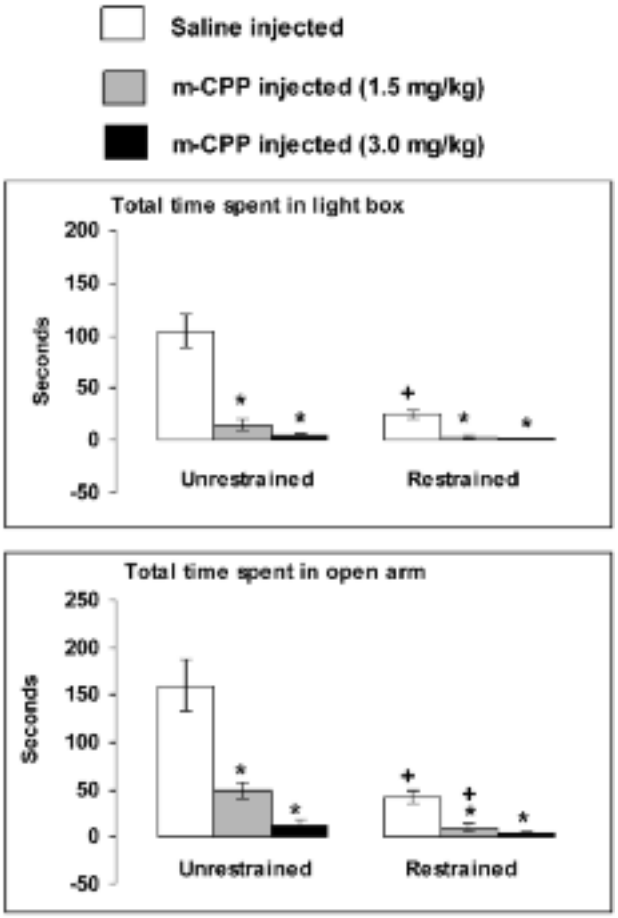

FIG. 2. — The anxiogenic-like effects of m-CPP in light-dark activity box and elevated plus maze in unrestrained and restrained animals. Values are means ± S.D. (n = 4). Significant differences by Newman-Keuls test : *P < 0.01 from saline injected animals, +P < 0.01 from respective unrestrained animals following two-way ANOVA.

of unrestrained and restrained animals. Saline injected restrained animals exhibited higher levels of 5-HT and 5-HIAA than their unrestrained counterparts.

Figure 4 shows the effects of m-CPP on dopamine (DA), DOPAC and homovanillic acid (HVA) levels in unrestrained and restrained animals. Data analyzed by two-way ANOVA showed significant effects of stress on DA levels ($F = 22.50$ $df = 1,18$ $p < 0.01$) and DOPAC ($F = 25.99$ $df = 1,18$ $p < 0.01$), while effects were not significant for HVA ($F = 1.99$ $df = 1,18$ $p > 0.05$). Effects of m-CPP were significant for DA ($F = 12.53$ $df = 2,18$ $p < 0.01$), DOPAC ($F = 21.87$ $df = 2,18$ $p < 0.01$) and HVA ($F = 6.1$ $df = 2,18$ $p < 0.05$). Interaction between stress and m-CPP were significant for DA ($F = 11.47$ $df = 1,18$ $p < 0.01$) and DOPAC ($F = 6.02$ $df = 1,18$ $p < 0.05$), and not significant for HVA ($F = 1.82$

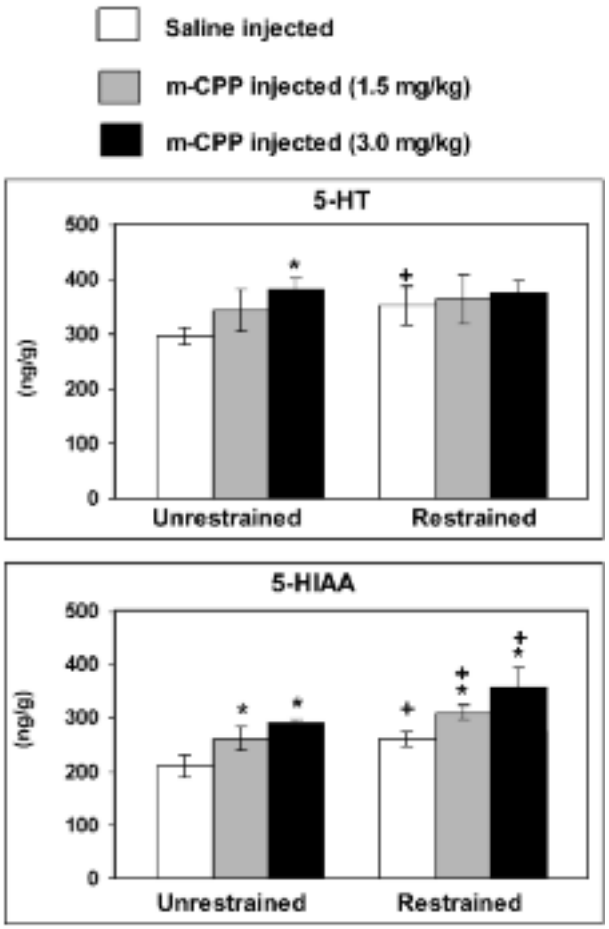

FIG. 3. — Effects of m-CPP administration on 5-HT and 5-HIAA levels in unrestrained and restrained animals. Values are means ± S.D. (n = 4). Significant differences by Newman-Keuls test : *P < 0.01 from saline injected animals, +P < 0.01 from respective unrestrained animals following two-way ANOVA.

df = 1,18 p > 0.05). Post-hoc analysis by Newman-Keuls test showed that administration of m-CPP at a dose of 1.5 mg/kg or 3.0 mg/kg decreased DA levels in unrestrained but not restrained animals. Administration of m-CPP alone at 3 mg/kg decreased DOPAC levels of unrestrained animals and at both 1.5 mg/kg and 3.0 mg/kg of restrained animals than their control counterparts. Saline injected restrained animals exhibited smaller brain levels of DA than their unrestrained counterparts. m-CPP treated restrained animals at a dose of 1.5 mg/kg and 3.0 mg/kg exhibited smaller levels of DOPAC respectively than m-CPP treated unrestrained animals.

Discussion

The aim of the present study was to determine the responsiveness of 5-HT-2C receptors following exposure to restraint stress. The present study shows that the effects of m-CPP on increasing 5-HIAA and decreasing DOPAC were more pronounced in restrained than unrestrained animals. Differences in the behavioral effects of m-CPP in restrained and unrestrained animals were not obvious in the present experimental paradigm because the behavioral effects of m-CPP were parallel to stress-induced behavioral deficits.

Previously, it has been reported that acute administration of m-CPP reduces locomotor activity in rats (Kennett & Curzon, 1988 ; Kennett *et al.*, 1997 : Kurt *et al.*, 2003 : Brus *et al.*, 2004). It is thought that ability of m-CPP to suppress spontaneous locomotor behavior in rats involves the 5-HT-2C receptor (Kennett & Curzon, 1988 : Fone & Topham, 2002). A selective 5-HT-2C receptor antagonist 6-Chloro-5-methyl-l-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl-carbomyl] indoline (SB-242084) has also been shown to suppress potently m-CPP-induced hypolocomotion (Kennett *et al.*, 1997). Furthermore, lesions of 5-HT neurons with 5,7 dihydroxy tryptamine potentiated the hypolocomotive effect of m-CPP, possibly by development of post-synaptic supersensitivity (Lucki *et al.*, 1989). Collectively evidence suggests that stimulation of 5-HT-2C receptors is involved in the hypolocomotive response to m-CPP. In the present study, differences in m-CPP-induced hypolocomotion in unrestrained and restrained animals (Fig. 1) can not be evaluated because restraint stress also decreased activity and the effects of m-CPP and restraint stress were additive.

It is generally accepted that increase 5-HT activity is responsible for anxiety. The total time spent in light compartment (light-dark activity) (Shimada *et al.*, 1995) and open arm (plus maze activity) (Lister, 1987 ; Pellow & File, 1986 ; Grieble *et al.*, 1994) has been considered as an index of anxiety in rats. Results from our laboratory have shown that a decrease in exploratory activity in a light compartment of light-dark activity box (Samad *et al.*, 2005) and open arm of plus maze activity (Samad *et al.*, 2002) following exposure to stress. Evidence suggests that m-CPP mediated anxiogenic-like effect by activation of central 5-HT-2C receptor (Fone *et al.*, 1996 ; Kennett *et al.*, 1997 ; Sills *et al.*, 1985 ; Freo *et al.*, 1992 ; Hackler *et al.*, 2007). In the present study, an anxiogenic-like behavior was also observed following m-CPP in unrestrained as well as restrained animals. A comparison of the hypolocomotive effects of m-CPP in restrained and unrestrained animals can not evaluated due to floor effect (Fig. 2).

The serotonin agonist m-CPP a metabolite of the antidepressant drug trazodone binds to various 5-HT

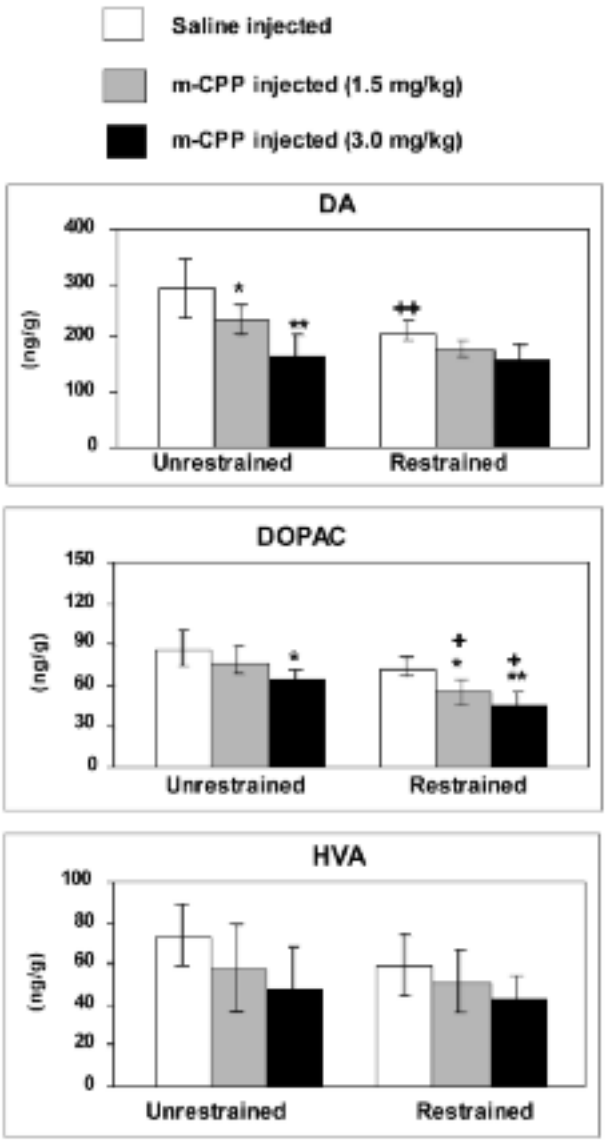

FIG. 4. — Effects of m-CPP administration on DA, DOPAC and HVA in unrestrained and restrained animals. Values are means ± S.D. (n = 4). Significant differences by Newman-Keuls test : *P < 0.05 and **p < 0.01 from saline injected animals, +P < 0.05 and ++p < 0.01 from respective unrestrained animals following two-way ANOVA.

receptors in rat brain and of these its binding affinity is more potent at 5-HT-2C receptors. m-CPP increases release of 5-HT (Hikiji *et al.*, 2004) via the stimulation of postsynaptic 5-HT-2C receptor (Gibson *et al.*, 1996).

Studies have shown that 2-h restraint stress increased 5-HT release and metabolism, in the whole brain (Samad *et al.*, 2006) and most brain regions (Haleem & Parveen, 1994) of rats, which is in agreement with the present results (Fig. 3).

The present study shows that administration of m-CPP increased 5-HT metabolism (Fig. 3) in both unrestrained and restrained animals. Restrained animals also exhibited an increase in 5-HT metabolism. m-CPP-induced increases of 5-HIAA were therefore more pronounced in restrained than unrestrained animals. It may be noted that m-CPP-induced increase of 5-HT did not occur in restrained animals suggesting attenuation of response to m-CPP in restrained animals.

The serotonergic system is known to inhibit dopamine neurotransmission at the level of the origin of dopamine system in the midbrain as well as in the terminal regions. This action of serotonin is due to the stimulation of 5-HT-2C receptors located on the somatodendritic and as well as nerve terminal of dopaminergic neuron (Pessia *et al.*, 1994 ; Millan *et al.*, 1998).

Dopamine plays an important role in fear and emotional response to stress. Puglisi-Allegra *et al.* (1991) have reported that restraint or foot shock (3-60 min) stress increased levels of DOPAC and HVA in the nucleus accumbens, while no significant changes occurred in dopamine concentration. Our data shows that 2-h restraint stress decreased dopamine levels in the whole brain, while DOPAC and HVA levels were not altered (Fig. 4).

Alex *et al.* (2005) have demonstrated that m-CPP decreased striatal dopamine release via the inhibition of nigrostriatal dopamine transmission. The present study shows a decrease in DA and DOPAC levels (Fig. 4) following the administration of m-CPP in unrestrained animals. This could be because of the inhibitory influence of m-CPP on the activity of dopaminergic neurons. The effects of m-CPP on the decreases of DOPAC were more pronounced in restrained than unrestrained animals.

In conclusion, the present study shows that behavioral effects of m-CPP such as hypolocomotion and anxiogenic-like effects were similar in restrained than unrestrained animals because restraint-stress also produced these behavioural effects. Differences in the behavioral effects of m-CPP in unrestrained and restrained animals can therefore not be evaluated due to a floor effect. m-CPP-induced increases of 5-HT concentration were smaller and m-CPP-induced decreases of DOPAC levels were more pronounced in restrained than unrestrained animals, suggesting that the responsiveness of 5-HT-2C receptors is altered following exposure to a 2-h restraint stress.

Acknowledgments

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