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Pre- and postsynaptic dopamine mechanism after the administration of apomorphine: relationship with restraint stress

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Abstract: Dopaminergic system and its D1 as well as D2 receptors are involved in the modulation of emotional behavior. The present study evaluated the role of dopaminergic activity after the single restraint stress (post-stress), a widely used learned helplessness model, by using the pharmacological manipulation through the apomorphine, an agonist for D1 and D2 receptors. Male albino Wistar rats were restrained for 2-h. 24-h after the termination of restraint period, apomorphine at doses of 0.05mg/kg and 0.25 mg/kg and saline 1 ml/kg was injected to unrestrained and restrained rats. Exploratory activity in an activity box was monitored 30 minutes after the drug and saline administration. Rats were decapitated 1-h post-injection to collect brain region (hypothalamus, hippocampus, cortex, midbrain, dorsal striatum and ventral striatum) for neurochemical analysis by high performance liquid chromatography with electrochemical detector (HPLC-EC). Administration of apomorphine at both doses increased exploratory activity of both unrestrained and restrained animals. Exploratory activity was smaller in restrained than unrestrained animals. Restrained animals injected with saline exhibited a decrease in dopamine (DA), 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) levels in various brain regions. The decreases were greater in apomorphine injected restrained than saline injected restrained animals.

Keywords: Apomorphine, restraint stress, HPLC-EC, dopaminergic activity.
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INTRODUCTION

Stressful events in the life of humans are associated with many mental disorders such as depression, anxiety and cardiovascular diseases[1,2]. Previous studies on experimental animals showed that an uncontrollable stress situation produced various neurochemical and behavioral deficits such as a decrease in food intake, growth rate and in exploratory activity[3,4]. Restraint stress has proven to be a very useful experimental procedure[5] as immobilization induces stress in the animals; this experimental procedure is useful for the examination of both central and peripheral mechanisms of stress induced deficits, as well as for studying effect of drugs on these deficits[6].

Apomorphine a dopamine (DA) agonist with selectivity towards both D1 and D2 receptors[7,8] is a CNS stimulant[9] with slightly higher affinity for D2-like dopamine receptors[7]. Apomorphine produces behavioral sensitization, characterized by a progressively greater increase in locomotor activity with repeated treatment[10,11] and immediate after the treatment[12]. It has been suggested that a common factor in the initiation of behavioral sensitization by drugs with different primary mechanisms of action is that they all decrease somatodendritic DA release[13,14]. At most doses apomorphine's postsynaptic action on DA receptors dominates, so it produces behavioral stimulation similar to amphetamine and cocain. Development of behavioral sensitization to apomorphine is related to the stimulation of DA D1 receptors[15] and an alteration in the synthesis of DA[16]. The expression of behavioral sensitization of psycho stimulants is associated with enhances extracellular DA levels in the nucleus accumbens beginning at least one week after discontinuing repeated psycho-stimulant administration[13,17].

The present study was designed to monitor the effectiveness of DA D2 receptors. Apomorphine a DA D2 receptor agonist[18] at doses 0.05 mg/kg or 0.25 mg/kg was injected 24-h after the termination of 2-h restraint stress to compare the responsiveness of DA D2 receptors. Stimulation of D2 receptors elicits an increase in motor activity[19]. Drug-induced increases of motor activity and other neurochemical effects of the drug are compared in restrained and unrestrained animals.

MATERIALS AND METHODS

Animals

Total 24 male albino Wistar rats (locally bred) weighing 170-220 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 and doses

Apomorphine-HCL (sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intraperitoneally at a dose of 0.05 mg/kg and 0.25mg/kg to the respective animals. Drug was freshly prepared before starting the experiment. Saline (0.9% NaCl solution; 1 ml/kg) was injected to control animals.

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 restrained for 2-h commencing between 9:00 – 11:00 h. Animals of the unrestrained group were left to their home cages during this time.

About 24-h after the termination of restraint stress animals further divided to six groups of 4 each were assigned as (i) saline unrestrained (1 ml/kg; 4 animals); (ii) saline restrained (1 ml/kg; 4 animals); (iii) apomorphine unrestrained (0.05 mg/kg; 4 animals); (iv) apomorphine restrained (0.05 mg/kg; 4 animals); (v) apomorphine unrestrained (0.25 mg/kg; 4 animals); (vi) apomorphine restrained (0.25 mg/kg; 4 animals) were injected accordingly with apomorphine and saline at 11:00-11:30. Motor activity was monitored in a home cage, 30 min after the saline and drug administration.

Animals were sacrificed 1-h after apomorphine or saline injection to collect brain samples. Brain regions (hippocampus, hypothalamus, cortex, midbrain, dorsal striatum and ventral striatum) dissected out were frozen at -70°C for neurochemical estimation by HPLC-EC.

Behavioral procedures

Restraining procedure

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[20]. 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 (26x26x26 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 minutes.

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+dentrate 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 DA and metabolites

Brain samples were homogenized as described previously[20]. DA, DOPAC and HVA levels were determined by HPLC-EC as described before[19]. A 5µ 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 Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu L-ECD-6A detector at an operating potential of 0.8 V.

Statistical analysis

Results are represented mean±SD. Behavioral and neurochemical data were analyzed by Two-way ANOVA. Individual comparisons were made by Newman-Keuls test.

RESULTS

Figure 1 shows the effect of administration of apomorphine on exploratory activity in home cage. Data analyzed by Two-way ANOVA showed significant effects of stress ($F=113.03$ $df=1, 18$ $p<0.01$) and apomorphine ($F=113.06$ $df=2, 18$ $p<0.01$) and a significant interaction between stress and apomorphine ($F=75.15$ $df=1, 18$ $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that restraint stress produced a large decrease in the scores of saline as well as apomorphine injected animals. Administration of apomorphine at doses 0.05 mg/kg or 0.25 mg/kg increased activity of unrestrained and restrained animals. Because of a large decrease in the activity of restrained animals producing a floor effect, exploratory activity increases at 0.05 mg/kg apomorphine were not significant in restrained animals.

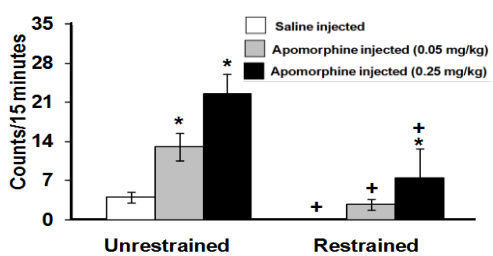

Figure 1: effect of administration of apomorphine on exploratory activity in home cage.

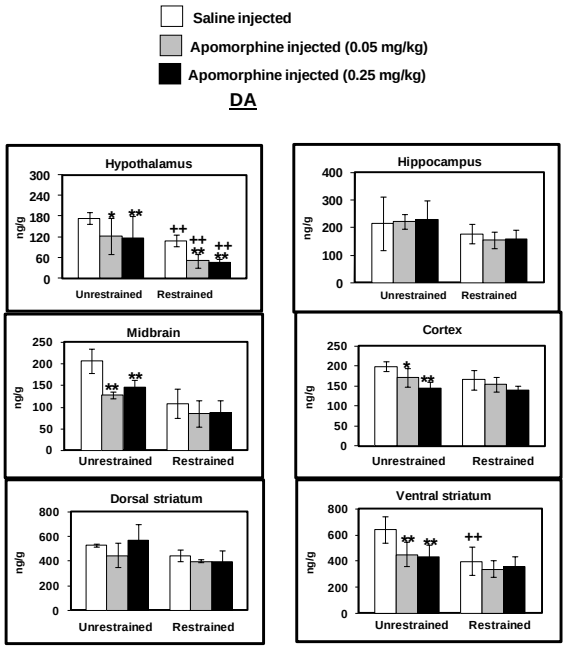

Figure 2: Effects of administration of apomorphine on DA levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals.

Figure 2 shows the effects of administration of apomorphine on DA levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals. Data on DA levels analyzed by Two Way ANOVA showed effect of stress was significant for hippocampus ($F=27.44$ $df=1,18$ $p<0.01$), midbrain ($F=40.33$ $df=1,18$ $p<0.01$), cortex ($F=6.40$ $df=1,18$ $p<0.05$) and ventral striatum ($F=15.16$ $df=1,18$ $p<0.01$), but not significant for hippocampus ($F=0.005$ $df=1,18$ $p>0.05$) and dorsal striatum ($F=3.07$ $df=1,18$ $p>0.05$). Effect of administration of apomorphine was significant for hypothalamus ($F=45.97$ $df=2,18$ $p<0.01$) hippocampus ($F=6.87$ $df=2,18$ $p<0.05$) midbrain ($F=17.37$ $df=2,18$ $p<0.01$), cortex ($F=14.30$ $df=2,18$ $p<0.01$) and ventral striatum ($F=9.94$ $df=2,18$ $p<0.01$) but not significant for dorsal striatum ($F=1.32$ $df=2,18$ $p>0.05$). Interaction between stress and apomorphine was significant for midbrain ($F=5.60$ $df=1,18$ $p<0.05$) and cortex ($F=7.55$ $df=1,18$ $p<0.05$), but not significant for hypothalamus ($F=0.24$ $df=1,18$ $p>0.05$), hippocampus ($F=0.45$ $df=1,18$ $p>0.05$), ventral striatum ($F=3.61$ $df=1,18$ $p>0.05$) and dorsal striatum ($F=0.39$ $df=1,18$ $p>0.05$). Post-hoc analysis by Newman-Keuls test showed that administration of apomorphine at doses 0.05 mg/kg or 0.25 mg/kg decreased DA levels of unrestrained and restrained animals in the hypothalamus, while decreased levels of unrestrained animals but not restrained animals in both midbrain and cortex. In the ventral striatum administration of apomoprhine at a dose of 0.05mg/kg and 0.25 mg/kg decreased DA levels of restrained than unrestrained animals. Saline injected as well as apomorphine injected restrained animals exhibited a decreased levels of DA in the hypothalamus and midbrain than unrestrained animals. An episode of 2-h restraint stress decreased DA levels of saline injected animals in the ventral striatum.

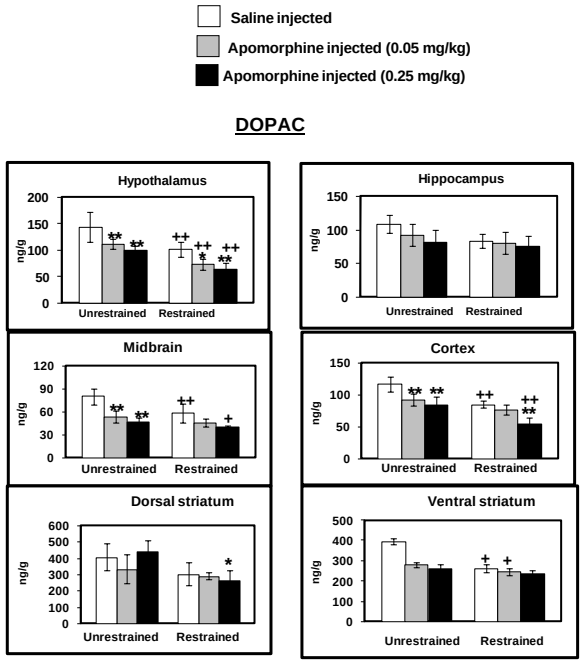

Figure 3: Effects of administration of apomorphine on DOPAC levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals.

Figure 3 shows the effects of administration of apomorphine on DOPAC levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals. Data on DOPAC levels analyzed by Two-way ANOVA showed that effects of stress were significant for hypothalamus ($F=37.38$ $df=1,18$ $p<0.01$), hippocampus ($F=22.34$ $df=1,18$ $p<0.05$), midbrain ($F=13.01$ $df=1,18$ $p<0.01$), cortex ($F=22.34$ $df=1,18$ $p<0.01$), dorsal striatum ($F=14.74$ $df=1,18$ $p<0.01$) and ventral striatum ($F=20.45$ $df=1,18$ $p<0.01$). Effects of administration of apomorphine were significant for hypothalamus ($F=22.74$ $df=2,18$ $p<0.01$), midbrain ($F=40.16$ $df=2,18$ $p<0.01$), cortex ($F=42.73$ $df=2,18$ $p<0.01$) and ventral striatum ($F=15.16$ $df=2,18$ $p<0.01$), but not significant for hippocampus ($F=4.30$ $df=2,18$ $p>0.05$) and dorsal striatum ($F=3.07$ $df=2,18$ $p>0.05$). Interaction between stress and apomorphine was significant for midbrain ($F=6.53$ $df=1,18$ $p<0.05$) and cortex

(F=13.24 df=1,18 p<0.01) but not significant for hypothalamus (F=2.18 df=1,18 p>0.05), hippocampus (F=2.57 df=1,18 p>0.05), dorsal striatum (F=0.39 df=1,18 p>0.05) and ventral striatum (F=3.61 df=1,18 p>0.05). Post hoc analysis by Newman-Keuls test showed that administration of apomorphine at doses 0.05 mg/kg or 0.25 mg/kg decreased DOPAC levels of unrestrained and restrained animals in the hypothalamus. In the midbrain and cortex administration of apomorphine (0.05 and 0.25 mg/kg) decreased DOPAC levels of unrestrained, while at the dose of 0.25 mg/kg decreased levels of DOPAC were observed in the cortex. In the dorsal striatum administration of apomorphine (0.25mg/kg) decreased levels of DOPAC of restrained animals, while in the ventral striatum of saline injected and apomorphine (0.05mg/kg) injected animals decreased DOPAC levels of restrained than unrestrained animals. In the hippocampus, hypothalamus and cortex saline injected as well as apomorphine injected restrained animals exhibited decreased levels of DOPAC in restrained than unrestrained animals.

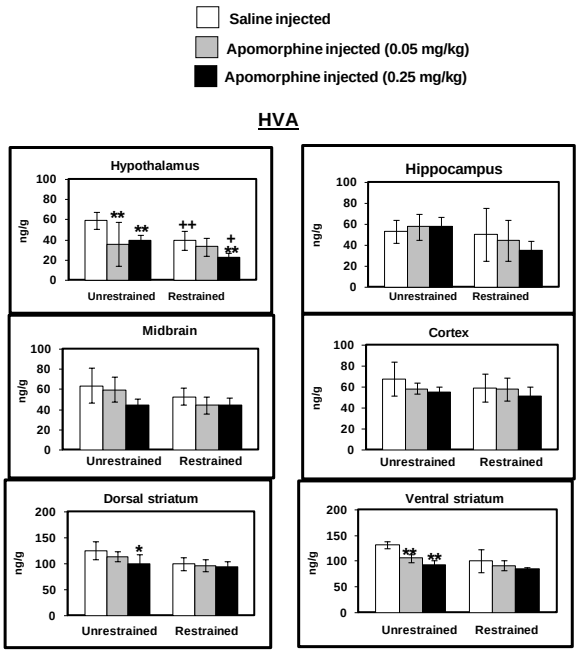

Figure 4: Effects of administration of apomorphine on HVA levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals.

Figure 4 shows the effects of administration of apomorphine on HVA levels in the hypothalamus, hippocampus, cortex, midbrain, ventral and dorsal striatum of restrained and unrestrained animals. Data on HVA levels analyzed by Two-way ANOVA showed significant effects of stress for hypothalamus (F=35.72 df=1,18 p<0.01), ventral striatum (F=11.49 df=1,18 p<0.01) and dorsal striatum (F=9.53 df=1,18 p<0.01), but not significant for hippocampus (F=4.10 df=1,18 p>0.05), midbrain (F=2.30 df=1,18 p>0.05) and cortex (F=1.44 df=1,18 p>0.05). Effects of administration of apomorphine were significant for hypothalamus (F=16.87 df=2,18 p<0.01) and ventral striatum (F=20.22 df=2,18 p<0.01), but not significant for hippocampus (F=0.20 df=2,18 p>0.05), midbrain (F=2.30 df=2,18 p>0.05), cortex (F=0.84 df=2,18 p>0.05) and dorsal striatum (F=4.10 df=2,18 p>0.05). Interaction between stress and apomorphine was significant for hypothalamus (F=4.5 df=1,18 p<0.05), midbrain (F=6.99 df=1,18 p<0.05) and ventral striatum (F=5.99 df=1,18 p<0.05), but not significant for hippocampus (F=1.93 df=1,18 p>0.05), cortex (F=1.44 df=1,18 p>0.05) and dorsal striatum (F=3.73 df=1,18 p>0.05). Post-hoc analysis by Newman-Keuls test showed that in the hypothalamus and ventral striatum administration of apomorphine at doses 0.05mg/kg and 0.25mg/kg decreased HVA levels of unrestrained animals. The decreases of HVA levels were also observed in the hypothalamus and dorsal striatum at a dose of 0.25mg/kg of apomorphine in restrained and unrestrained animals respectively. In the hypothalamus saline injected and apomorphine (0.25mg/kg) injected animals exhibited a decrease of HVA levels in restrained than unrestrained animals. Differences by Newman-Keuls test were not significant in the midbrain and cortex.

DISCUSSION

The aim of the present study was to determine the responsiveness of DA D2 receptor following exposure to 2-h restraint stress. Results show that apomorphine-induced increases of motor activity were smaller in restrained than unrestrained animals. Administration of decreased DA metabolism in various brain regions of restrained as well unrestrained animals. Apomorphine-induced decreases of DA metabolism were smaller in restrained than unrestrained animals.

Many studies have reported that systemic injection of dopaminergic drugs result in profound changes in the behavior of animals. In agreement with previous data[21], the systemic administration of apomorphine increased motor activity at both doses examined (Figure 1). Unlike amphetamine and methamphetamine, which act as indirect dopamine agonists, direct acting agonists are more selective and include dopamine D2 preferring agonist bromocriptine[22], mixed D1/D2 agonist apomorphine[23-25] and mixed D2/D3 agonist quinpirol[26,27]. From perspective of sensitized

mesocorticolimbic dopamine neurotransmission, a sensitized locomotor response following administration of apomorphine (Figure 1) could occur as a result of an increase in post-synaptic receptor sensitivity or as a result of sub-sensitivity of the inhibitory autoreceptors[13].

An additional and important finding of the present study is that apomorphine-induced behavioral stimulation was smaller in restrained than unrestrained animals (Figure 1), suggesting decrease in the responsiveness of postsynaptic D2 receptor following exposure to restraint stress. The fact that exposure to potent stressors such as swim stress, foot shock and restraint stress enhances dopamine activity in the mesocorticolimbic system[28-30] suggests that stress and apomoprhine-induced additive and excessive enhancement of mesocorticolimbic dopamine neurotransmission is involved in the greater behavioral sensitization (Figure 1) observed in apomorphine injected animals exposed to immobilization 1 h later.

The dopaminergic system is suggested to be the neural substrate for defensive behavior. It may contribute to generation and elaboration of defensive behavior in response to threat or danger[31]. It has been reported that systemic administration of apomorphine inhibits DA neuronal impulse flow[32] in the substantia nigra. Carey et al[33] have also reported that, DA turnover is decreased following the administration of apomorphine. It has been reported that the density of D1 receptor is significantly higher in the nucleus accumbens and striatum of rats that did not become helpless after stress, compared to rats that developed learned helplessness[34]. Because the upregulation of D1 receptor may refer to the lower transmission of dopaminergic system, it suggests that lower activity of this system may be associated with adaptive or protective role in the prevention of escape deficits after exposure to inescapable stress. These results provide new insights into the possible role of dopaminergic activity in the coping behavior or learning ability in the stress. Furthermore apomorphine at doses 0.25 mg/kg and 1 mg/kg) decreased DOPAC and HVA levels in the caudate putaman and nucleus accumbens septi[35]. In the present study administration of apomorphine decreased DA metabolism in the hypothalamus, midbrain, cortex and ventral striatum of unrestrained animals (Figures 2, 3, 4). Apomorphine-induced decreases of DA metabolism were smaller in the hypothalamus, midbrain, cortex and ventral striatum of restrained than unrestrained animals (Figures 2, 3, 4) suggesting a decrease in the effectiveness of D2 presynaptic receptors.

In conclusion, the present study suggests that an episode of 2-h restraint stress decreased the functional activity of DA. Results suggested that the effectiveness of presynaptic and postsynaptic D2 receptors is decreased following exposure to single restraint stress.

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