

Attenuation of Unpredictable Chronic Mild Stress induced deficits in rats by Apomorphine

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Abstract

Objective: To investigate the effects of repeated doses of apomorphine in rats exposed to unpredictable chronic mild stress.

Methods: The experimental case-control study was conducted in March 2011 at the Department of Biochemistry, University of Karachi, and comprised albino-wistar rats. The experimental animals were exposed to a schedule of unpredictable chronic mild stress, while control animals were left in the cages. Animals of stressed and unstressed group were administered with apomorphine at dose of 1.0 mg/kg repeatedly. Daily food intake and activity in familiar environment were monitored to observe the anorexic effects and behavioural sensitisation after apomorphine administration.

Results: The 24 rats in the study were divided into two groups. Apomorphine-induced anorexia was seen more in stressed animals ($p < 0.05$). Apomorphine increased motor activity monitored in a familiar environment and apomorphine-induced behavioural sensitisation was more potentiated in animals previously exposed to unpredictable chronic mild stress as they showed more cage crosses after 2nd and 3rd ($p < 0.05$) and 4th to 6th ($p < 0.01$) injections compared to the stressed rats with saline. These animals also exhibited hyperactive response in open field apparatus after 1st and 6th injection ($p < 0.01$) compared to the saline-injected stressed rats.

Conclusion: Repeated administration of apomorphine at 1.0 mg/kg dose alleviated the behavioural deficits of unpredictable chronic mild stress.

Keywords: Unpredictable chronic mild stress, Depression, Locomotors activity, Behavioural sensitisation, Dopamine agonist, Apomorphine. (JPMA 66: 1541; 2016)

Introduction

Apomorphine, a dopamine or dopamine agonist (DA) with selectivity towards D1 and D2 receptors,¹ is a central nervous system (CNS) stimulant.² The repeated administration of direct or indirect dopamine receptor agonists produces a progressive increase in the acute behavioural effects of drugs, an effect known as behavioural sensitisation.^{3,4} Psychostimulant substances, several of which are abused by humans, are generally known to yield a sensitisation effect when they are administered repeatedly to animal subjects.⁵ The behavioural sensitisation produced by apomorphine is characterised by a progressively greater increase in locomotor activity with repeated treatment⁴ or it can be produced shortly after the treatment.⁶ A study suggested that behavioural sensitisation is initiated by different drugs with different primary mechanisms of action, but these drugs share a common point that they all decrease somatodendritic DA release.³ The doses of apomorphine from 0.5-5mg/kg increase the locomotor activity and rodents exhibit stereotyped behavioural, sniffing, licking

and gnawing. The increased locomotor activity is sometimes expressed as climbing and turning behaviour.^{4,7-10}

Chronic exposure to stressful life events is an established risk factor for the development of many psychological conditions in humans, including major depression. Various animal models of depression were used to study behavioural and neurochemical responses to challenge drugs.^{11,12} The first chronic stress model — unpredictable chronic mild stress (UCMS) — of depression was developed in 1981.¹³ Also in 1987, researchers developed UCMS protocol which included a variety of low-grade stressors administered over a long period of time.¹⁴ UCMS is used as an animal model of depression. Most of the effects can be reversed by selective serotonin reuptake inhibitors (SSRIs),^{14,15} illustrating a strong predictive validity. In rodents, UCMS also has good face validity as it can elicit depression-like symptoms such as a lack of sucrose preference.^{14,16}

Locomotor sensitisation to apomorphine is dependent on time interval between injection and testing. It has been suggested that apomorphine-induced hyperactivity is induced because of desensitisation of dopamine autoreceptors.¹⁷

Stress-induced changes in brain reward circuits increase

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the sensitivity to the reinforcing properties of drugs, thereby increasing the motivation to use drugs compulsively.¹⁸ Exposure to electric foot-shock stress also increased the subsequent reinforcing efficacy of heroin¹⁹ and morphine²⁰ in rats. Previous works²¹⁻²⁶ have reported that exposure to an episode of 2-h immobilisation stress decreased 24-h cumulative food intake and body weight in rats. The animals exhibited anxiety/depression like behaviour in light dark transition test, elevated plus maze test^{23,27,28} and forced swimming test.^{28,29} The deficits in food intake and other behaviours no longer persisted upon repeated immobilisation.^{21,24,26,30} It was suggested that repeated exposure to the same stressor produces adaptive changes that led to behavioural tolerance.²⁷ Drug-induced hypersensitivity of motivational circuitry is suggested to mediate an increase in drug "wanting," shifting recreational drug use to pathological abuse displayed by addicts.³¹ Although sensitisation and reinstatement involve overlapping neuronal circuitry, neurotransmitters and their receptors, but the involvement of sensitisation in reinstated drug-seeking behaviour remains controversial.^{32,33} Behavioural sensitisation, however, remains a useful model for determining neural basis of addiction and neuro-adaptations associated with the behavioural sensitisation are considered initial step in the drug addiction.

Since CNS stimulants could be used for the treatment of depression, we planned the present study to monitor the effects of apomorphine in UCMS model of depression. The study will help to understand the interaction of stress with addiction and anorexia at behavioural and neurochemical level, it can demonstrate whether uncontrollable life event stress potentiates addiction and anorexia. Moreover, use of addictive drugs produces depression or stress-induced depression leads to addiction.

Material and Methods

The experimental case-control study was conducted in March 2011 at the Department of Biochemistry, University of Karachi. After getting approval from the institutional review board, locally-bred male albino-wistar rats weighing 180-220g were purchased from Aga Khan University, Karachi, that were housed individually under 12-h light and dark cycle and controlled room temperature (25±2) with free access to cubes of standard rodent diet and water for at least 3 days before experimentation.

Apomorphine-hydrochloride (HCl) (Sigma, St. Louis, USA) was dissolved in saline (0.9% sodium chloride [NaCl]) and injected intraperitoneally at a dose of 1.0 mg/kg to the respective group animals. Drug was freshly prepared

before starting the experiment. Saline (0.9% NaCl solution; 1ml/kg) was injected to control animals.

The rats were equally divided into two groups: Unstressed animals and UCMS animals. Animals of unstressed group did not receive any type of stressor during the 10-day period and remained in their cages with all accessibilities e.g. food, water, controlled temperature and environment.

UCMS group was exposed to a schedule of different unpredictable mild stress for 10-day duration and afflicted with different types of stressors during this time span.³⁴ After 10 days, both groups were subdivided into two groups each: Saline and Apomorphine. This resulted in a total of four groups: Unstressed-Saline, Unstressed-Apomorphine, UCMS-Saline and UCMS-Apomorphine injected animals. Animals were administered accordingly with apomorphine (1.0 mg/kg) or saline (1.0 mg/ml) on alternate days for the next 12 days. Daily food intake and activity in familiar environment of Skinner's box were monitored 24hr after apomorphine administration. Exploratory activity was monitored 24hr after first and last (6th) apomorphine administration in an open field.

Behavioural Assessments

Round-the-clock food intake was monitored. A weighed amount of food was placed in the hooper in the cage of each animal. Intake was monitored by weighing the food left in the hooper of the cage after the required time.

The assessment of locomotor activity and exploration in a familiar environment was done by home cage activity test. The apparatus used was a rectangular Perspex activity cage and consisted of small square area (26x26x26 cm) with a saw-dust covered floor. Testing was done in a quiet room under weight and light.³⁵ The animals were placed in the home cage for habituation 15 minutes before monitoring the activity. Number of crossing across the boxes were monitored for 10 minutes.

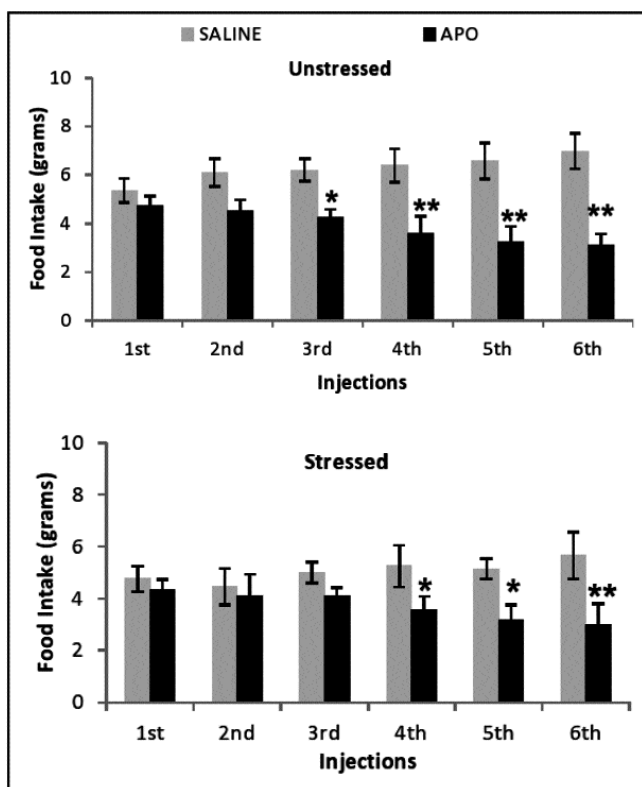
The assessment of locomotor activity and exploration in a novel environment was done by open field activity test. The test consisted of measuring the activity of rats in an open novel space, from which escapee was prevented by a surrounding wall.³⁶

SPSS 17 was used statistical analysis. Values were presented as means ± standard deviation (SD). Data on drug administration of unstressed and stressed rats was analysed by three-way analysis of variance (ANOVA) (repeated measures design). Post-hoc comparison was done by Newman-Keuls test. Values of $p < 0.05$ were considered significant.

Results

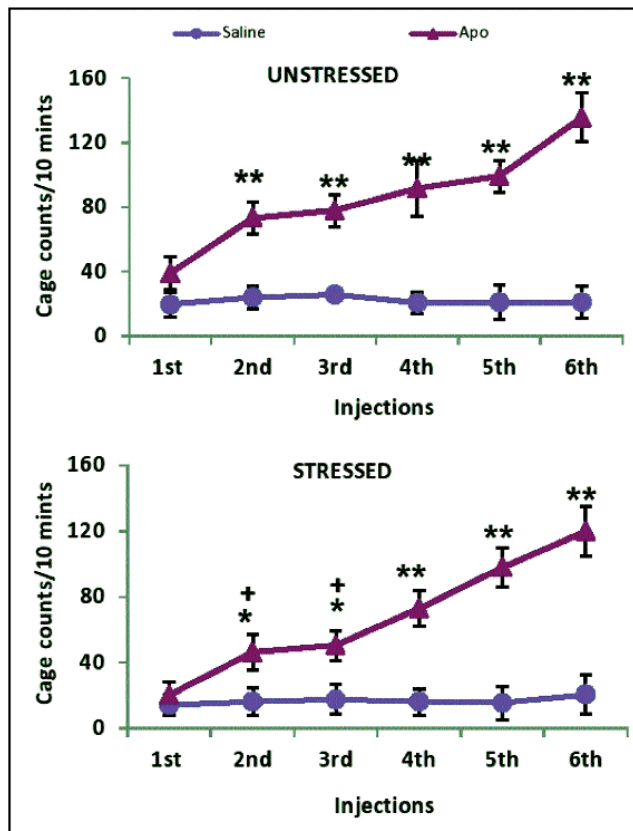
There were 24 rats in the study that were divided into two groups of 12(50%) each. Significant effects of apomorphine in terms of food intake were noticed ($F=23.07$; $df= 1, 21$; $p<0.01$) and the interaction among stress, apomorphine and repeated monitoring ($F=6.807$; $df= 5, 17$; $p<0.01$). However, the effects of stress ($F=0.716$; $df= 1, 21$) and repeated monitoring ($F=0.912$; $df= 5, 17$; $p>0.01$) were found to be non-significant. Apomorphine decreased food intake after 3rd ($p<0.05$), 4th, 5th and 6th injection ($p<0.01$) in unstressed rats compared to unstressed saline injected controls. In UCMS rats, apomorphine decreased food intake after 3rd, 4th ($p<0.05$) and 6th ($p<0.01$) administration than similarly treated saline injected rats (Figure-1).

Significant effects of stress ($F=17.14$; $df= 1, 21$; $p<0.01$), apomorphine ($F=392.42$; $df= 1, 21$; $p<0.01$) and repeated monitoring ($F=22.58$; $df= 5, 17$; $p<0.01$) on activity (cage



UCMS: Unpredictable chronic mild stress. ANOVA: Analysis of variance. APO: Apomorphine. SD: Standard deviation

Figure-1: Effects of apomorphine administration on food intake in male rats exposed to UCMS. Values are means \pm SD ($n=6$) as monitored 24 hours after each drug administration. Significant differences by Newman-Keuls test: * $p<0.05$. ** $p<0.01$ from respective saline injected controls; following three-way ANOVA (repeated measures design).

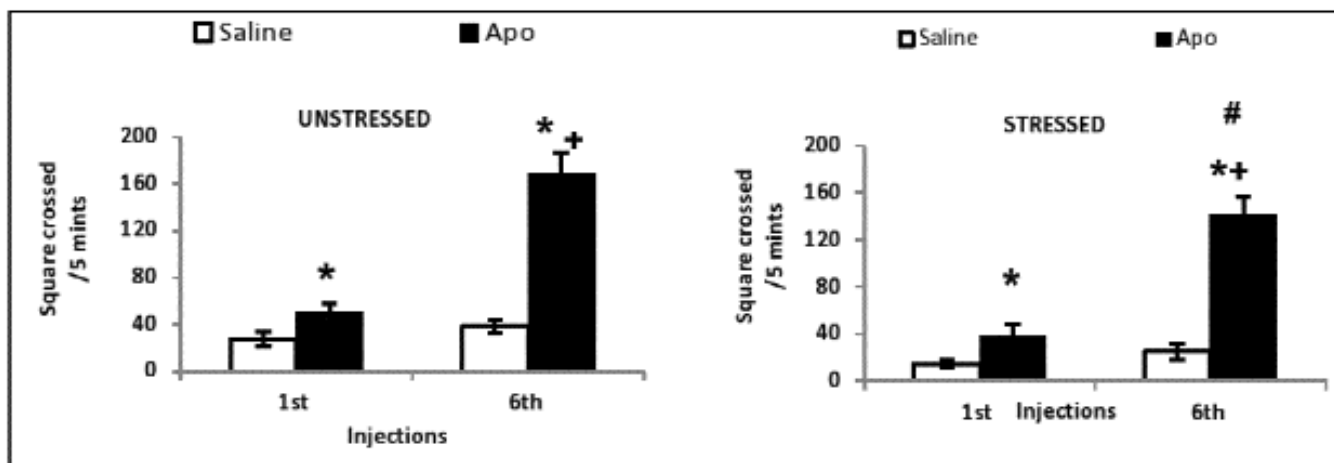


UCMS: Unpredictable chronic mild stress. ANOVA: Analysis of variance. SD: Standard deviation.

Figure-2: Effects of apomorphine administration on activity in activity box of male rats exposed to UCMS. Values are means \pm SD ($n=6$) as monitored 24 hours after each drug administration. Significant differences by Newman-Keuls test: * $p<0.05$, ** $p<0.01$ from respective saline injected rats; + $p<0.05$, ++ $p<0.01$ from similarly injected unstressed controls following three-way ANOVA (repeated measures design).

crossing) in a familiar environment was seen in cage-crossings. However the interaction among stress, apomorphine and repeated monitoring ($F=161.15$; $df= 5, 17$; $p<0.01$) were also significant. Apomorphine increased ($p<0.01$) cage crossings in the unstressed animals after 2nd to 6th injections compared to unstressed saline injected animals. The cage crossings also increased in stressed apomorphine-injected rats after 2nd and 3rd ($p<0.05$) injection as well as ($p<0.01$) after 4th to 6th compared to stressed saline injected controls. Injections of apomorphine after 2nd and 3rd day decreased ($p<0.05$) activity (cage crossing) in activity box of stressed animals compared to similarly injected unstressed animals on the same days (Figure-2).

Significant effects of stress ($F=19.97$; $df= 1, 21$; $p<0.01$), apomorphine ($F=479.15$; $df= 1, 21$; $p<0.01$), repeated



UCMS: Unpredictable chronic mild stress. ANOVA: Analysis of variance. SD: Standard deviation. APO: Apomorphine

Figure-3: Effects of apomorphine administration on activity in open field of male rats exposed to UCMS. Values are means \pm SD (n=6) as monitored 24 hours after 1st and 6th day administration. Significant differences by Newman-Keuls test: * $p < 0.01$ from respective saline injected rats; + $p < 0.01$ from similarly injected rats from 1st administration; # $p < 0.01$ from similarly injected unstressed rats following three-way ANOVA (repeated measures design).

monitoring ($F=57.45$; $df= 1, 21$; $p < 0.01$) and interaction among stress, apomorphine and repeated monitoring ($F=238.2$; $df= 1, 21$; $p < 0.01$) was seen in terms of open field activity. Apomorphine increased ($p < 0.01$) activity in open field (square crossing) after 1st and 6th injections of unstressed as well as stressed group animals compared to respective similarly treated saline-injected controls. Apomorphine increased ($p < 0.01$) activity after 6th injections in unstressed and stressed groups of animals compared to respective similarly treated apomorphine animals after the 1st injection. UCMS apomorphine-injected rats showed decreased ($p < 0.01$) activity after 6th injection compared to similarly injected unstressed rats (Figure-3).

Discussion

The study was designed to monitor the effects of unpredictable stress on the behavioural effects of apomorphine. Apomorphine, an agonist at dopamine D1/D2 receptors, elicits sensitisation on repeated administration. Sensitisation is a well-known component of psychostimulant-induced addiction. Uncontrollable stress may lead to depression while prevalence of depression is greater in addicts than normal population. In previous studies, repeated restraint and UCMS has been reported to increase and to decrease the locomotor response to DA, respectively.³⁷⁻³⁹ UCMS has been used as an animal model of depression and these effects of UCMS can be altered by antidepressant agents,^{14,15,40} illustrating a strong predictive validity. Many studies have reported that systemic injections of dopaminergic drugs results in

profound changes in the behaviour of animals.⁴¹ Results from a previous study³⁴ revealed that exposure to UCMS induced hypophagic condition (decreased food intake) in rats. This decrease in food intake could be due to the anhedonia (inability to feel pleasure) produced by chronic stress. Chronic stress-mediated alteration in hypothalamic-pituitary-adrenal (HPA) axis is root cause of hypophagia. Reduction in sucrose preference in rats following UCMS has also been reported.⁴² Exposure to unpredictable stressors have resulted in inducing significant changes in behavioural parameters, such as altered locomotive and explorative behaviour, a decline in food intake, water intake and sexual activity.⁴³ A study also suggested that chronic unpredicted mild stress-induced behavioural deficits in experimental animals could be used effectively as animal model of depression.⁴⁴ UCMS decreased locomotor activity as monitored in familiar environment in rats. This could be due to the decrease in serotonergic function resulting in the development of depressive symptoms.⁴⁵ In our study, repeated administration of apomorphine revealed a significant increase in locomotor activity of stressed as well as unstressed animals. Apomorphine-induced locomotive activity were smaller in UCMS animals than unstressed animal. Our study revealed that repeated administration of apomorphine to rats previously exposed to UCMS, resulted in a decrease in food intake. Previously it has been reported that systematic administration of dopaminergic drugs induces significant changes in the behaviours of animal model. The present study reports that apomorphine-induced changes in

behavioural parameters were smaller in UCMS animals than unstressed animals. In agreement with previous study⁴⁶ the repeated administration of apomorphine increased motor activity in familiar as well as in novel environments at dose 1.0 mg/kg. This effect might be due to the involvement of DA D1/D2 receptors, as the drug we used has a well-known mechanism of action, a D1/D2 agonist.⁴⁷

Conclusion

Repeated administration of apomorphine at dose of 1.0 mg/kg attenuated deficits induced by UCMS. Chronic stress is known to produce alteration in the serotonin neurotransmitter system. UCMS decreases food intake and produces anxiety and depression-like symptoms in rats. Administration of apomorphine increased activities in familiar and exploratory environment of unstressed as well as in stressed animals.

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