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Restraint-induced behavioral deficits are attenuated or impaired by pre- or post-injection of apomorphine: A context-based study

Huma Ikram[1]*, Shoaib Ahmed[1] and Darakhshan Jabeen Haleem[1,2]
[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan
[2]Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: Apomorphine, a psycho stimulant, has neuroprotective effects due to its ability to decrease oxidative stress. Stress-induced dopaminergic dysfunction might lead to posttraumatic stress disorder, depression and related disorders. This dopaminergic dysfunction is more pre-dominant in basal ganglia and prefrontal cortex. Targeting of this dysfunction by psychostimulants, involves elevating dopamine in these brain regions and reduction of stress. On the other hand, stress itself can aggravate addictive effects to psycho stimulants. Present study was therefore designed to monitor the role of apomorphine in the attenuation of stress-induced behavioral deficits. Rats were exposed to 2hr restraint stress either before or after the apomorphine administration, to monitor effects of apomorphine administration on stress-induced behavioral deficits. Stress-induced decreases in food intake, growth rate and elevated plus maze activity were exacerbated if apomorphine was experienced during restraint stress. Conversely, these behavioral deficits were attenuated if apomorphine was experienced after restraint stress. It shows that apomorphine, if experienced during restraint stress, produces greater behavioral deficits, while the same were attenuated in rats receiving apomorphine after the termination of restraint stress. Results suggest that apomorphine and possibly the other CNS stimulants may help to cope stress by attenuating stress-induced behavioral deficits, if experienced after stress.

Keywords: Apomorphine, stress, behavioral deficits, elevated plus maze, food intake, growth rates.

INTRODUCTION

Neurodegeneration, trauma, ischemia and related disorders are caused by the oxidative stress-induced neuronal cell death. Apomorphine is believed to mediate its protective effects through which could be inhibited by dopamine D_4 antagonist U101958. Apart from its agonistic activity towards D_4 receptors, antioxidant effects of apomorphine are also mediated by the inhibition of glutamate-induced reactive oxygen species production and blockade of cGMP-operated Ca^{2+} channels (Ishige *et al*., 2001).

Exposure to stress causes dopaminergic dysfunction and also could lead to other psychiatric disorders (Howes *et al*., 2017). It has been reported that exposure to stress causes an increase in basal extracellular dopamine levels in the medial prefrontal cortex (Mokler *et al*., 2007). In responses to pain stress, nucleus accumbens and caudate-putamen dopamine D2 receptor mRNA levels correlate well with cocaine reinstatement suggesting that variations in the pain experience and responses to pain involve D2 receptor-mediated neurotransmission in the basal ganglia (Lee *et al*., 2018; Scott *et al*., 2006). Stress also can aggravate addictive effects to psychostimulants. There is considerable literature reporting correlation of addiction vulnerability and lifetime exposure to unpredictable and uncontrollable stressors. An escalation and initiation of drug self-administration also increases following exposure to stress (Sinha, 2008).

Apart from dopamine, serotonin plays a key role in the pathophysiology of both stress and addiction. Brain centers regulating anxiety and fear include hippocampus, amygdala, frontal cortex, median and dorsal raphe nuclei, show that reuptake and release of 5-HT along with the sensitivity of post- and presynaptic 5-HT receptors are affected by various acute and repeated/chronic stressors (Zhao *et al*., 2018; Puglisi-Allegra and Andolina, 2015; Chaouloff, 2000). An altered 5-HT metabolism and post- and presynaptic 5-HT receptors sensitivity is these regions is also involved in mediating the effects of drugs of abuse including alcohol, opioids and psychostimulants. Transition to compulsive drug use is attained by the serotonergic adaptations which render the nervous system susceptible drug abuse (Kuypers *et al*., 2018; Müller and Homberg, 2015).

It has been reported previously that apomorphine-induced behavioral sensitization is not produced in rats co-injected with buspirone (Ikram and Haleem, 2011). It was suggested that a decrease in the responsiveness of somatodendritic $5\text{-}HT_{1A}$ receptors could increase the inhibitory influence of 5-HT on the activity of dopaminergic neurons, resulting in the attenuation of apomorphine-induced behavioral sensitization. An increased availability of 5-HT also facilitates the

*Corresponding author: e-mail: huma_biochemist@yahoo.com

adaptation to restraint stress. While adaptation to restraint stress could also be achieved by the selective activation of postsynaptic 5-HT_{1A} receptors and it could serve as a potential treatment strategy for stress and depression (Zhou *et al*., 2014). Present study was therefore designed to further investigate the role of somatodendritic 5-HT_{1A} receptors in the attenuation of stress-induced behavioral deficits by apomorphine, as somatodendritic 5-HT_{1A} receptors are involved in the regulation of 5-HT synthesis and release (Araragi *et al*., 2013). Since single episode of restraint stress decreases availability of 5-HT (Lee *et al*., 2018; Ikram *et al*., 2017) and single injection of apomorphine increases the same (Ikram *et al*., 2018), it was therefore suggested that the stress-induced behavioral deficits will be smaller or will not occur in rats injected with apomorphine.

MATERIALS AND METHODS

Experimental animals

Study was carried out on locally bred male Albino Wistar rats (150-250g) purchased from HEJ Research Institute of Chemistry. Rats were kept individually in specially designed cages in a quiet room with free access to water and cubes of standard rodent diet for at least 1 week before starting the experiment so that rats adopt the environment.

Drugs and doses

Apomorphine-HCl purchased from Sigma (St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intraperitonelly at a dose of 1.0 mg/kg to the rats. Drug was freshly prepared before starting the experiment. Controls were injected with saline (0.9% NaCl) in volumes of 1ml/kg.

Experimental protocol

Experiment No. 1: Restraining before apomorphine administration

Twenty four male rats were randomly divided into four groups, each containing 6 rats each: (i) unrestraint-saline, (ii) unrestraint-apomorphine, (iii) restraint-saline, and (iv) restraint-apomorphine injected rats. Rats of restraint groups were restrained (in restraining tubes) for 2hr, while rats of unrestraint groups were kept in their home cages. Rats were exposed to daily restraint stress sessions of 1hr each from day 1-6. 1hr post restraint stress, rats were injected with saline (1ml/kg) or apomorphine (1mg/kg) respectively. Food intake and growth rates were monitored daily. Activities in elevated plus maze were monitored post 1st and 6th apomorphine injection.

Experiment No. 2: Restraining after apomorphine administration

Twenty four male rats were randomly divided into four groups, each containing 6 rats each: (i) saline-unrestraint (ii) saline-restraint (iii) apomorphine- unrestraint and (iv) apomorphine-restraint rats. Rats were daily injected with saline (1ml/kg) or apomorphine (1mg/kg) respectively. 1hr post injection, rats of restraint groups were restrained (in restraining tubes) for 2hr, while rats of unrestraint groups were kept in their home cages. Rats were exposed to daily restraint stress sessions of 1hr each from day 1-6. Food intake and growth rates were monitored daily. Activities in elevated plus maze were monitored post 1st and 6th apomorphine injection.

Restraining procedure

Restraint stress was produced by placing rats in adjustable (8" long and 2" diameter) plexiglas tubes with air holes in the front, top and back. Restraining was achieved by allowing the rats to enter into tubes and pressing their bodies gently to let them fit into the tube. Tail was passed through the lid hole at the back and that adjustable lid was fixed to prevent any movement of rat. Method was essentially same as described elsewhere (Weinberg *et al*., 2007).

Behavioral assessment

Monitoring food intake and growth rates

Cumulative food intakes (g) were determined by taking the difference of food given on day 1, between 8:00 and 9:00h and food left next day and every day (between 8:00 and 9:00h). Body weights were also monitored at the same time and change in body weights were calculated (body weight on monitoring day / body weight on preceding day)×100 as reported previously (Ikram *et al*., 2017).

Elevated plus maze activity

Procedure was same as described earlier (Ikram *et al*., 2017). Elevated plus-maze is a cross shaped maze that has two open arms and two close arms enclosed by sides, but with an open roof. The entire maze is elevated 50cm above the floor. Rodent's unconditioned aversion to light and open spaces contribute to its effectiveness as a test for anxiety. Test involves placement of animal in the centre of maze and observing the number of entries and time spent in open/fear inducing arm.

Brain dissection

After decapitation, skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. Hippocampus was collected by using brain microdissection as described earlier (Ikram and Haleem, 2017) and collected samples were immediately stored at -70°C for neuro chemical estimations using High performance liquid chromatography with electrochemical detection (HPLC-EC)

Neurochemical estimations by HPLC-EC

HPLC-EC determination was carried out as described earlier (Sheikh *et al*., 2015). A 5µ Shim-pack ODS

separation column of 4.0mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH 2.9 at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu LEC 6A detector at an operating potential of +0.8V.

Ethical approval

All experiments were performed in accordance with approval from Institutional Ethical Approval Committee (IEAC).

STATISTICAL ANALYSIS

Results are represented as means ± S.D. Statistical analysis was performed by three-way ANOVA (SPSS ver 17.0). Post hoc comparison of groups was performed by Tukey's test. Values of $p<0.05$ were considered significant.

RESULTS

Fig. 1 shows effects of apomorphine, restraint stress and their interaction on daily food intake. Analysis of the data on effects of restraint stress on food intake before apomorphine administration (fig. 1a), by three-way ANOVA showed significant effects of apomorphine (df= 1,120; F= 64.118; p= 0.0001), restraint stress (df= 1,120; F= 875.60; p= 0.0001) and repeated monitoring (df= 5,120; F= 63.67; p= 0.0001). Interactions of apomorphine*restraint stress (df= 1,120; F= 56.74; p= 0.0001), apomorphine*repeated monitoring (df= 5,120; F= 52.90; p= 0.0001), restraint stress*repeated monitoring (df= 5,120; F= 14.46; p= 0.0001) and apomorphine*restraint stress*repeated monitoring (df= 5,120; F= 19.70; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased ($p<0.01$) food intake by saline-restraint- as well as apomorphine-restraint rats as compared to respective unrestraint rats, from day 2 to day 4 but not afterwards. Apomorphine-restraint rats showed greater ($p<0.01$) food intake as compared to saline-restraint rats, on day 5 and 6 but not before that.

Analysis of the data on effects of restraint stress on food intake after apomorphine administration (fig. 1b), by three-way ANOVA showed significant effects of apomorphine (df= 1,120; F= 891.59; p= 0.0001), restraint stress (df= 1,120; F= 59.98; p= 0.0001) and repeated monitoring (df= 5,120; F= 59.75; p= 0.0001). Interactions of apomorphine*restraint stress (df= 1,120; F= 72.12; p= 0.0001), apomorphine*repeated monitoring (df= 5,120; F= 54.35; p= 0.0001), restraint stress*repeated monitoring (df= 5,120; F= 19.69; p= 0.0001) and apomorphine*restraint stress*repeated monitoring (df= 5,120; F= 14.93; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased ($p<0.01$) food intake by saline-restraint rats as compared to respective unrestraint rats, from day 2 to day 4 but not afterwards. Food intake in apomorphine-restraint rats was also decreased ($p<0.01$) as compared to respective unrestraint rats, from day 2 to day 6 and were also decreased ($p<0.01$) as compared to respective saline injected rats from day 4 to day 6.

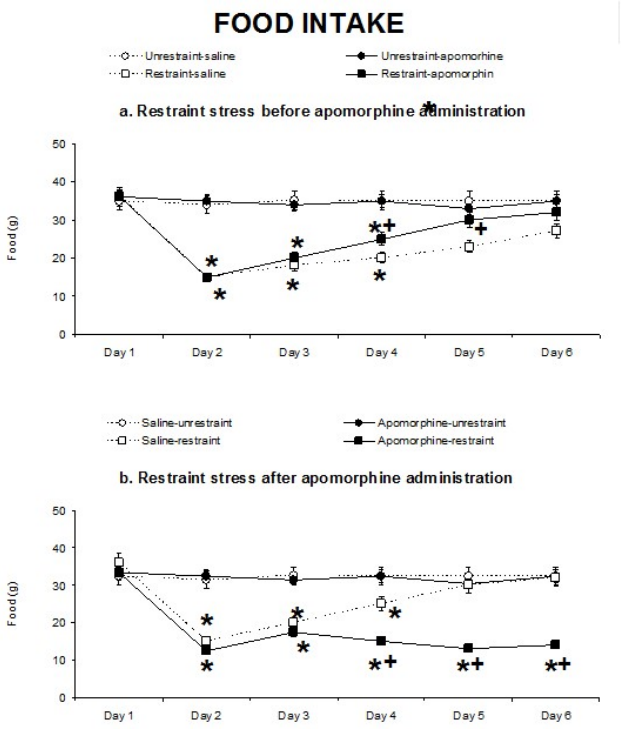

Fig. 1: Effects of restraint-stress, apomorphine and their interaction on food intake. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective unrestraint controls; +p<0.01 as compared to respective saline injected rats, following three-way ANOVA (repeated measure design).

Fig. 2 shows effects of apomorphine, restraint stress and their interaction on daily growth rates. Analysis of the data on effects of restraint stress on growth rates before apomorphine administration (fig. 2a), by three-way ANOVA showed significant effects of apomorphine (df= 1,120; F= 130.00; p= 0.0001), restraint stress (df= 1,120; F= 2160.78; p= 0.0001) and repeated monitoring (df= 5,120; F= 160.57; p= 0.0001). Interactions of apomorphine*restraint stress (df= 1,120; F= 84.80; p= 0.0001), apomorphine*repeated monitoring (df= 5,120; F= 105.53; p= 0.0001), restraint stress*repeated monitoring (df= 5,120; F= 26.62; p= 0.0001) and apomorphine*restraint stress*repeated monitoring (df= 5,120; F= 17.96; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased ($p<0.01$) growth rates by saline-restraint- as well as apomorphine-restraint rats as compared to respective unrestraint rats, from day 2 to day 4 but not afterwards.

Analysis of the data on effects of restraint stress on growth rates after apomorphine administration (fig. 2b), by three-way ANOVA showed significant effects of apomorphine (df= 1,120; F= 311.50; p= 0.0001), restraint stress (df= 1,120; F= 187.20; p= 0.0001) and repeated monitoring (df= 5,120; F= 231.23; p= 0.0001). Interactions of apomorphine* restraint stress (df= 1,120; F= 122.12; p= 0.0001), apomorphine* repeated monitoring (df= 5,120; F= 151.97; p= 0.0001), restraint stress*repeated monitoring (df= 5,120; F= 38.32; p= 0.0001) and apomorphine*restraint stress*repeated monitoring (df= 5,120; F= 11.46; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased (p<0.01) growth rates by saline-restraint rats as compared to respective unrestraint rats, from day 2 to day 4 but not afterwards. Growth rates in apomorphine-restraint rats was also decreased (p<0.01) as compared to respective unrestraint rats, from day 2 to day 6 and were also decreased (p<0.01) as compared to respective saline injected rats from day 4 to day 6.

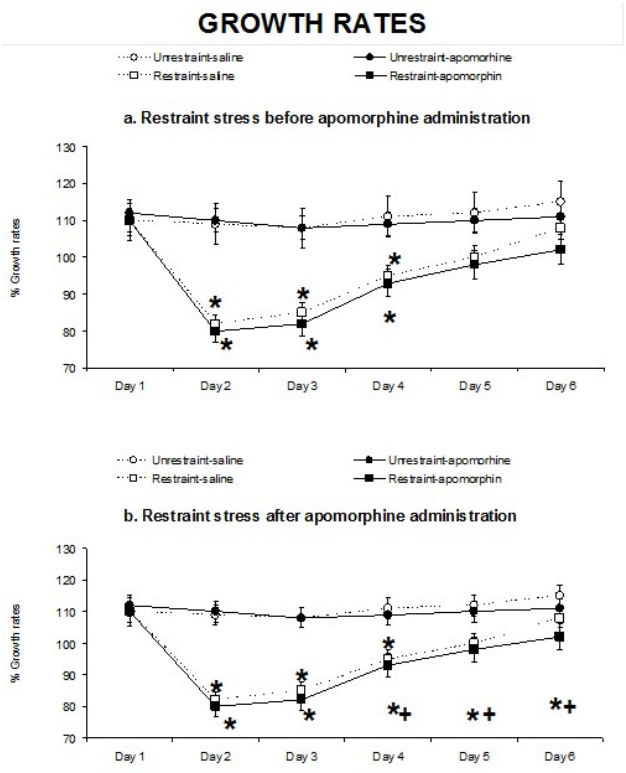

Fig. 2: Effects of restraint-stress, apomorphine and their interaction on growth rates. Values are means ± SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective unrestraint controls; +p<0.01 as compared to respective saline injected rats, following three-way ANOVA (repeated measure design).

Fig. 3 shows effects of apomorphine, restraint stress and their interaction on elevated plus maze activity. Analysis of the data on effects of restrain stress on activities in elevated plus maze (entries in open arm) before apomorphine administration (fig. 3a), by three-way ANOVA showed significant effects of apomorphine (df= 1,40; F= 51.12; p= 0.0001), restraint stress (df= 1,40; F= 21.98; p= 0.0001) and repeated monitoring (df= 1,40; F= 53.46; p= 0.0001). Interactions of apomorphine*restraint stress (df= 1,40; F= 21.97; p= 0.0001), apomorphine*repeated monitoring (df= 1,40; F= 21.54; p= 0.0001), restraint stress*repeated monitoring (df= 1,40; F= 67.21; p= 0.0001) and apomorphine*restraint stress*repeated monitoring (df= 1,40; F= 23.65; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased (p<0.01) entries by unrestraint-apomorphine as well as restraint-apomorphine injected rats (post 1st injection) rats as compared to their respective saline injected controls. Entries by unrestraint-apomorphine injected rats (post 6th injection) were increased (p<0.01) as compared to their respective post 1st injection values. However these were attenuated (p<0.01) in restraint-apomorphine injected rats (post 6th injection) as compared to respective unrestraint rats.

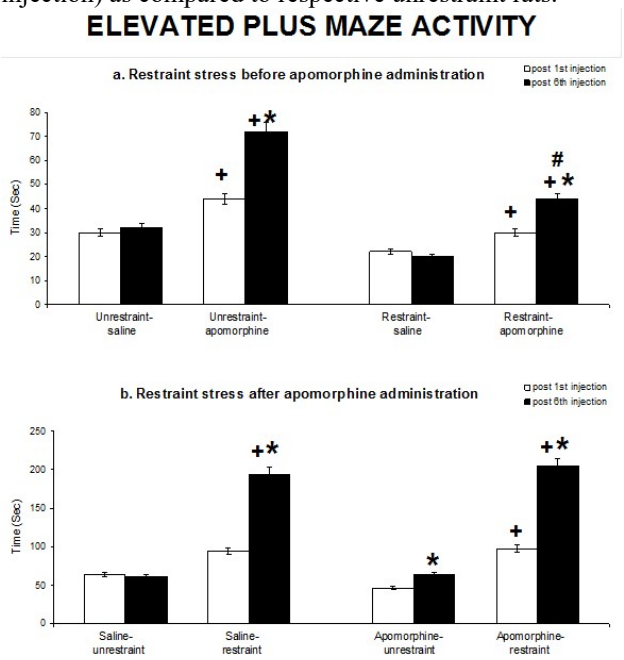

Fig. 3: Effects of restraint-stress, apomorphine and their interaction on elevated plus maze activity. Values are means ± SD (n=6). fig 3a: Significant differences by Tukey's test: *p<0.01 as compared to respective values post first injection; +p<0.01 as compared to respective unrestraint rats; #p<0.01 as compared to respective saline injected rats, following three-way ANOVA (repeated measure design). fig. 3b: Significant differences by Tukey's test: *p<0.01 as compared to respective values post first injection; +p<0.01 as compared to respective saline injected rats, following three-way ANOVA (repeated measure design).

Analysis of the data on effects of restraint-stress on activities in elevated plus maze (entries in open arm) after apomorphine administration (fig. 3b), by three-way ANOVA showed significant effects of apomorphine (df=

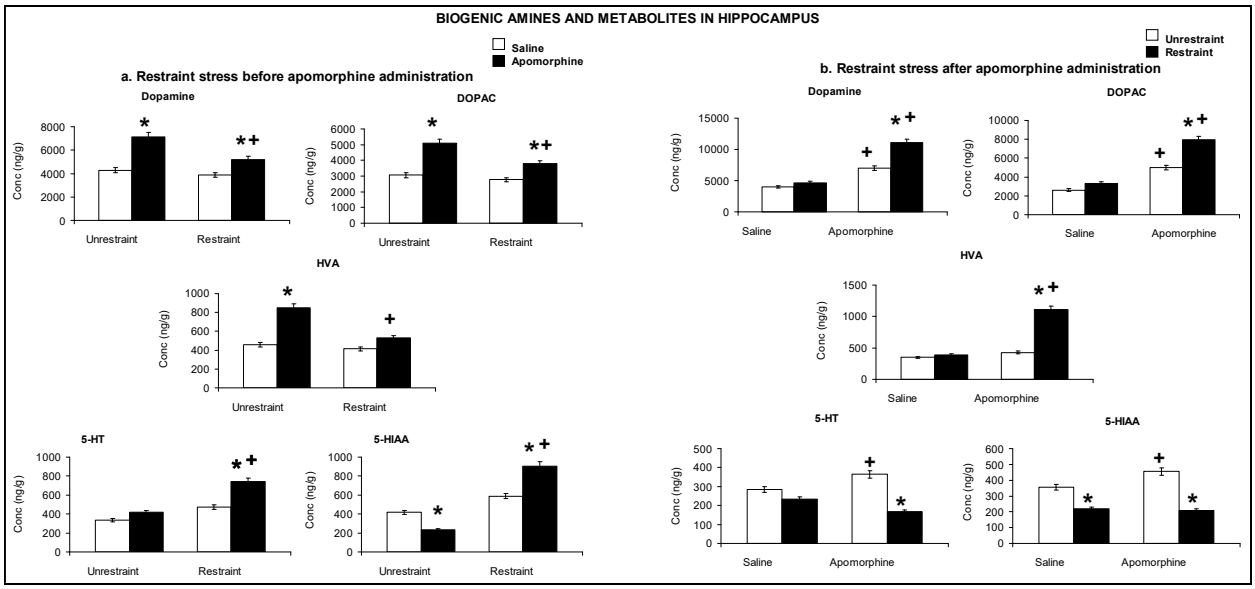

Fig. 4: Effects of restraint-stress, apomorphine and their interaction on levels of biogenic amines and metabolites in rat hippocampus. Values are means ± SD (n=6). fig. 4a: Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected rats; +p<0.01 as compared to respective unrestraint controls, following two-way ANOVA. fig. 4b: *p<0.01 as compared to respective unrestraint controls; +p<0.01 as compared to respective saline injected rats, following two-way ANOVA.

1,40; F= 62.15; p= 0.0001), restraint stress (df= 1,40; F= 45.21; p= 0.0001) and repeated monitoring (df= 1,40; F= 12.35; p= 0.0001). Interactions of apomorphine* restraint stress (df= 1,40; F= 39.12; p= 0.0001), apomorphine* repeated monitoring (df= 1,40; F= 55.21; p=0.0001), restraint stress* repeated monitoring (df=1,40; F=21.06; p=0.0001) and apomorphine* restraint stress*repeated monitoring (df=1,40; F=16.23; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased (p<0.01) entries by saline-restraint (post 1st injection) and apomorphine-restraint (post 1st as well as post 6th injection) rats as compared to their respective unrestraint controls. Entries by saline-restraint and apomorphine-unrestraint rats (post 6th injection) were significantly increased (p<0.01) as compared to their respective post 1st injection values.

Fig. 4 shows effects of apomorphine, restraint stress and their interaction on biogenic amines and metabolites in rat hippocampus. Analysis of the data on effects of restraint stress on biogenic amines and metabolites before apomorphine administration (fig. 4a), by two-way ANOVA showed that for dopamine levels, effects of restraint stress (df= 1,20; F= 1601.97; p= 0.0001), apomorphine (df= 1,20; F= 5075.30; p= 0.0001) and interaction between the two (df= 1,20; F= 67.81; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased dopamine levels in unrestraint-apomorphine as well as restraint-apomorphine injected rats compared to their respective saline injected controls. While levels of dopamine in restraint-apomorphine injected rats were decreased (p<0.01) as compared to unrestraint-apomorphine injected controls. Analysis of the data on DOPAC levels (fig. 4a) by two-way ANOVA showed that effects of restraint stress (df= 1,20; F= 7490.42; p= 0.0001), apomorphine (df= 1,20; F= 2714.45; p= 0.0001) and interaction between the two (df= 1,20; F= 302.37; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased DOPAC levels in unrestraint-apomorphine as well as restraint-apomorphine injected rats compared to their respective saline injected controls. While levels of DOPAC in restraint-apomorphine injected rats were decreased (p<0.01) as compared to unrestraint-apomorphine injected controls. Analysis of the data on HVA levels (fig. 4a) by two-way ANOVA showed that effects of restraint stress (df=1,20; F= 377.74; p=0.0001), apomorphine (df=1,20; F= 736.28; p= 0.0001) and interaction between the two (df=1,20; F= 215.85; p=0.0001) were all significant. Post hoc analysis by Tukey's test showed increased HVA levels in unrestraint-apomorphine injected rats compared to their respective saline injected controls. While levels of HVA in restraint-apomorphine injected rats were decreased (p<0.01) as compared to unrestraint-apomorphine injected controls.

Analysis of the data on 5HT levels (fig. 4a), by two-way ANOVA showed that effects of restraint stress (df= 1,20; F= 618.33; p= 0.0001), apomorphine (df= 1,20; F= 360.89; p= 0.0001) and interaction between the two (df= 1,20; F= 103.16; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased 5-HT levels in restraint-apomorphine injected rats compared to both respective saline injected- and unrestraint- controls. Analysis of the data on 5-HIAA levels (fig. 4a) by two-way ANOVA showed that effects of restraint stress (df=

1,20; F= 884.62; p= 0.0001), apomorphine (df= 1,20; F= 501.85; p= 0.0001) and interaction between the two (df= 1,20; F= 128.92; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased 5-HIAA levels in unrestraint-apomorphine injected rats as compared to their respective saline injected controls. Post hoc analysis by Tukey's test showed increased 5-HIAA levels in restraint-apomorphine injected rats as compared to both respective saline injected- and unrestraint- controls.

Figure 4b shows effects of restraint stress on biogenic amines and metabolites after apomorphine administration. Analysis of the data on dopamine levels by two-way ANOVA showed that effects of apomorphine (df= 1,20; F= 884.62; p= 0.0001), restraint stress (df= 1,20; F= 501.85; p= 0.0001) and interaction between the two (df= 1,20; F= 128.92; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased ($p<0.01$) dopamine levels in unrestraint-apomorphine- as well as restraint-apomorphine injected rats as compared to respective saline injected controls. While levels of dopamine were high ($p<0.01$) in restraint-apomorphine injected rats as compared to unrestraint-apomorphine injected rats. Analysis of the data on DOPAC levels (fig. 4a) by two-way ANOVA showed that effects of apomorphine (df= 1,20; F= 567.87; p= 0.0001), restraint stress (df= 1,20; F= 354.25; p= 0.0001) and interaction between the two (df= 1,20; F= 36.29; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased ($p<0.01$) DOPAC levels in unrestraint-apomorphine- as well as restraint-apomorphine injected rats as compared to respective saline injected controls. While levels of DOPAC were greater ($p<0.01$) in restraint-apomorphine injected rats as compared to unrestraint-apomorphine injected rats. Analysis of the data on HVA levels (fig. 4a) by two-way ANOVA showed that effects of apomorphine (df= 1,20; F= 675.87; p= 0.0001), restraint stress (df= 1,20; F= 398.25; p= 0.0001) and interaction between the two (df= 1,20; F= 87.86; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed increased ($p<0.01$) HVA levels in restraint-apomorphine injected rats as compared to respective saline injected- as well as respective unrestraint rats.

Analysis of the data on 5-HT levels (fig. 4a) by two-way ANOVA showed that effects of apomorphine (df= 1,20; F= 883.87; p= 0.0001), restraint stress (df= 1,20; F= 856.87; p= 0.0001) and interaction between the two (df= 1,20; F= 192.98; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased ($p<0.01$) 5-HT levels in restraint-apomorphine injected rats as compared to respective unrestraint controls. While levels of 5-HT in unrestraint-apomorphine injected rats were increased ($p<0.01$) as compared to respective saline injected controls. Analysis of the data on 5-HIAA levels (fig. 4a) by two-way ANOVA showed that effects of apomorphine (df= 1,20; F= 657.39; p= 0.0001), restraint stress (df= 1,20; F= 387.28; p= 0.0001) and interaction between the two (df= 1,20; F= 72.98; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed decreased ($p<0.01$) 5-HIAA levels in both restraint-saline- as well as restraint-apomorphine injected rats as compared to their respective unrestraint controls. While levels of 5-HIAA in unrestraint-apomorphine injected rats were increased ($p<0.01$) as compared to respective saline injected controls.

DISCUSSION

Results from the present experiment show that first episode of 2hr restraint stress decreased food intake and growth rates in saline injected animals. The decreases of both growth rates and food intake were attenuated following third and fourth episodes of daily 2hr restraint stress, suggesting adaptation to repeated restraint stress schedule and are in accordance with those reported previously from our laboratory (Haleem *et al*., 2013). Hypophagia induced by apomorphine injection in unrestrained rats was not significant. Results from the present study show that after first injection of apomorphine, restrained-induced behavioral deficits did not occur. It has also been reported that alcohol-dependent rats exhibited potentiation of stress-induced behavioral deficits in (Serrano *et al*., 2018). It is very interesting that both apomorphine and alcohol could attenuate hypophagia after first episode of 2h restraint stress but potentiate it afterwards (Ikram *et al*., 2013). Decreased food intake and growth rates were more potentiated in restrained-apomorphine injected rats when apomorphine was injected before restraint stress. conversely, apomorphine potentiated adaptation to stress-induced behavioral deficits, when injected after restraint stress. When injected before stress, potentiation of stress-induced behavioral deficits by apomorphine suggests that apomorphine can alleviate stress if experienced during stress.

Dopaminergic system is involved in the pathophysiology of stress and psychiatric disorders. This may cause an increase in the activity of dopaminergic neurons of ventral tegmental area through stress-induced neurochemical alterations in the ventral hippocampus (Belujon and Grace, 2015). A down-regulation of vesicular monoamine transporter 2 in dopaminergic regions is also important for mediating the effects of chronic stress. Exposure to chronic stress induces variations in brain morphology including a decrease in volumes of hippocampal grey matter (Gianaros *et al*., 2007). In the present study, adaptation to repeated restrained-stress normalized dopamine metabolism in hippocampus. In comparison to unrestraint-apomorphine injected rats, dopamine metabolism was decreased in restrained-apomorphine injected animals when apomorphine was injected after

restraint stress, suggesting that apomorphine by decreasing dopamine metabolism may attenuate stress-induced behavioral deficits. While potentiated dopamine metabolism was observed in hippocampus of restrained-apomorphine injected animals when apomorphine was injected before restraint stress, suggesting the involvement of increased dopamine metabolism in the potentiation of stress-induced behavioral deficits in these animals.

Saal *et al* (2003) have suggested that both addictive drugs and stress could induce plasticity of the dopaminergic neurons at the excitatory synapses. This synaptic plasticity may be a key factor in interaction of addiction and stress. In the present study, apomorphine, when injected before restraint stress, potentiated the increased dopamine metabolism in the restrained-apomorphine injected animals, thereby impaired adaptation to behavioral deficits in these animals.

We also observed an increased 5-HT metabolism in hippocampus samples of rats adapted to repeated restraint stress. Enhanced 5-HT functions are reported to be associated with adaptation to repeated restraint stress (Barr and Forster, 2011) and a deficiency of 5-HT may impair this adaptation and leads to depression (Cowen and Browning, 2015). In the present study, apomorphine decreased 5-HT metabolism in hippocampus of rats experiencing restraint-stress after apomorphine injection. Carta and Tronci (2014) have reported an increased 5-HT turnover rate by apomorphine, apart from stimulation of dopamine receptors stimulation by it. We therefore suggest that in apomorphine injected rats, due to increased release of dopamine, there might be an inhibitory effect of dopamine on 5-HT release when apomorphine was injected before restraint stress, suggesting an increased sensitivity of somatodendritic 5-HT_{1A} receptors resulting in decreased availability of 5-HT. This 5-HT metabolism was elevated in restrained-apomorphine injected animals if apomorphine was injected after restraint stress, thereby suggesting that increased 5-HT metabolism in these animals may have an important role in facilitationg adaptation to restraint stress-induced behavioral deficits.

CONCLUSION

In conclusion, the present results suggest that apomorphine and possibly the other CNS stimulants may help to cope stress by attenuating stress-induced behavioral deficits. The finding that stress-induced behavioral deficits were smaller in rats exposed to repeated restraint stress before apomorphine injection, supports previous findings from our laboratory that addictive drugs increase the sensitivity of somatodendritic 5-HT_{1A} receptors (Ikram and Haleem, 2011) to decrease the availability of 5-HT in the terminal region, while adaptation to stress involves a decrease in the sensitivity of somatodendritic 5-HT_{1A} receptors resulting in an increase in availability of 5-HT in the terminal region. It may be relevant that when apomorphine was injected before restraint stress, stress-induced behavioral deficits might be potentiated due to increase in the sensitivity of somatodendritic 5-HT_{1A} receptors that tend to release dopaminergic neurons from the inhibitory influence of 5-HT. The finding that apomorphine if experienced during stress, potentiates stress-induced behavioral deficits together with the results that if experienced after stress, attenuates stress-induced behavioral deficits, tend to show that drug of abuse may be effective for the treatment but not prevention of stress-induced depression.

REFERENCES

Araragi N, Mlinar B, Baccini G, Gutknecht L, Lesch KP and Corradetti R (2013). Conservation of 5-HT1A receptor-mediated auto inhibition of serotonin (5-HT) neurons in mice with altered 5-HT homeostasis. *Front. Pharmacol.,* **4**(1): 97.

Barr JL and Forster GL (2011). Serotonergic neuro-transmission in the ventral hippocampus is enhanced by corticosterone and altered by chronic amphetamine treatment. *Neuroscience,* **182**(1): 105-114.

Belujon P and Grace AA (2015). Regulation of dopamine system responsivity and its adaptive and pathological response to stress. *Proc. Biol. Sci.,* **282**(1805): 20142516.

Carta M and Tronci E (2014). Serotonin system implication in l-DOPA-Induced Dyskinesia: From animal models to clinical investigations. *Front. Neurol.,* **5**(1): 78.

Chaouloff F (2000). Serotonin, stress and corticoids. *J. Psychopharmacol.,* **14**(2): 139-151.

Cowen PJ and Browning M (2015). What has serotonin to do with depression? *World Psychiatry,* **14**(2): 158-160.

Haleem DJ, Ikram H, Haider S, Parveen T and Haleem MA (2013). Enhancement and inhibition of apomorphine-induced sensitization in rats exposed to immobilization stress: Relationship with adaptation to stress. *Pharmacol. Biochem. Behav.,* **112**(1): 22-28.

Howes OD, McCutcheon R, Owen MJ and Murray R (2016). The role of genes, stress and dopamine in the development of schizophrenia. *Biol. Psychiatry,* **81**(1): 9-20.

Ikram H and Haleem DJ (2011). Attenuation of Apomorphine-Induced Sensitization by Buspirone. *Pharmacol. Biochem. Behav.,* **99**(3): 444-450.

Ikram H and Haleem DJ (2017). Repeated treatment with reserpine as an animal model of depression. *Pak. J. Pharm. Sci.,* **30**(3): 897-902.

Ikram H, Mirza B and Haleem DJ (2017). Neurochemical and behavioral effects of green tea (*Camellia sinensis*) as observed in animals exposed to restraint stress. *Pak. J. Pharm. Sci.,* **30**(2): 487-492.

Ikram H, Zakir R and Haleem DJ (2018). Effects of single administration of apomorphine on memory and monoamine metabolism: A dose related study. *Pak. J. Pharm. Sci.,* **31**(2): 439-445.

Ishige K, Chen Q, Sagara Y and Schubert D (2001). The activation of dopamine D4 receptors inhibits oxidative stress-induced nerve cell death. *J. Neurosci.,* **21**(16): 6069-6076.

Kuypers KPC, de la Torre R, Farre M, Xicota L, de Sousa Fernandes Perna EB, Theunissen EL, Ramaekers JG (2018). Depressive mood ratings are reduced by MDMA in female polydrug ecstasy users homozygous for the l-allele of the serotonin transporter. *Sci. Rep.,* **8**(1): 1061.

Lee B, Shim I, Lee H and Hahm DH (2018). Berberine alleviates symptoms of anxiety by enhancing dopamine expression in rats with post-traumatic stress disorder. *Korean J. Physiol. Pharmacol.,* **22**(2): 183-192.

Mokler DJ, Torres OI, Galler JR and Morgane PJ (2007). Stress-induced changes in extra cellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatally malnourished rats. *Brain Res.,* **1148**(1): 226-233.

Müller CP and Homberg JR (2015). The role of serotonin in drug use and addiction. *Behav. Brain Res.,* **277**(1): 146-192.

Puglisi-Allegra S and Andolina D (2015). Serotonin and stress coping. *Behav. Brain Res.,* **277**(1): 58-67.

Saal D, Dong Y, Bonci A and Malenka RC (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron,* **37**(4): 577-582.

Scott DJ, Heitzeg MM, Koeppe RA, Stohler CS, Zubieta JK (2006). Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. *J. Neurosci.,* **26**(42): 10789-10795.

Serrano A, Pavon FJ, Buczynski MW, Schlosburg J, Natividad LA, Polis IY, Stouffer DG, Zorrilla EP, Roberto M, Cravatt BF, Martin-Fardon R, de Fonseca FR and Parsons LH (2018). Deficient endocannabinoid signaling in the central amygdala contributes to alcohol dependence-related anxiety-like behavior and excessive alcohol intake. *Neuropsychopharmacology*, **43**(1): 1840-1850.

Sheikh S, Ikram H and Haleem DJ (2015). Behavioral deficits in rats following acute administration of glimepiride: Relationship with brain serotonin and dopamine. *Pak. J. Pharm. Sci.,* **28**(4): 1181-1186.

Sinha R (2008). Chronic stress, drug use, and vulnerability to addiction. *Ann. NY Acad. Sci*., **1141**(1): 105-130.

Weinberg MS, Girotti M, Spencer RL (2007). Restraint-induced fra-2 and c-fos expression in the rat forebrain: relationship to stress duration. *Neuroscience,* **150**(2): 478-486.

Zhao J, Niu C, Wang J, Yang H, Du Y, Wei L and Li C (2018). The depressive-like behaviors of chronic unpredictable mild stress-treated mice, ameliorated by Tibetan medicine Zuotai: Involvement in the hypothalamic-pituitary-adrenal (HPA) axis pathway. *Neuropsychiatr. Dis. Treat.,* **14**(1): 129-141.

Zhou J, Cao X, Mar AC, Ding YQ, Wang X, Li Q and Li L (2014). Activation of postsynaptic 5-HT1A receptors improve stress adaptation. *Psychopharmacology* (Berl), **231**(10): 2067-2075.