EFFECTS OF APOMORPHINE ON LOCOMOTIVE ACTIVITY AND MONOAMINE METABOLISM: A DOSE RELATED STUDY

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ABSTRACT

We have monitored dose dependent effects of apomorphine on motor activity and monoamine metabolism. Behavioral sensitization and craving, which develop upon repeated treatment with dopamine receptor agonist apomorphine, are major limitations of the therapeutic use of apomorphine in Parkinson's patients. Effects of single (intraperitoneal) injection of apomorphine at different doses (i.e., 1.0, 2.0 & 4.0 mg/kg) on exploration in a novel environment (open field) and locomotion in a familiar environment (home cage) were investigated. Results show significantly enhanced activity in home cage (monitored 5min post injection) in a dose dependent manner. However, no significant influence of apomorphine on exploration of open field was observed in the present study (monitored 15min and 40min post injection). Animals were decapitated 1 hr post apomorphine injection and whole brains of animals were collected and stored at -70^{o}C. Biogenic amines (i.e., 5-Hydroxytryptamine and dopamine) and metabolites (i.e., Dihydroxyphenylacetic acid, Homovanillic acid & 5-Hydroxyindoleacetic acid) were estimated by reverse phase High Performance Liquid Chromatography with electrochemical detector (HPLC-EC). Effect of low (1.0mg/kg) dose of apomorphine was found to be non-significant on 5-Hydroxytryptamine (5-HT), 5-Hydroxyindoleacetic acid (5-HIAA) and dopamine (DA) levels. Moderate (2.0 mg/kg) dose of drug increased (p<0.05) levels of Homovanillic acid (HVA). Whereas, high (4.0 mg/kg) dose of apomorphine decreased Dihydroxyphenylacetic acid (DOPAC) levels. Results could be helpful in elucidating the effect of apomorphine at different doses and its implication for extending therapeutics in Parkinson's and related disorders.

Keywords: Apomorphine, CNS stimulant, serotonin, dopamine, behavioral sensitization.

INTRODUCTION

Apomorphine is a psychostimulant having agonistic activity for both D_1 and D_2 receptors with slightly higher affinity for D_2-like dopamine receptors (Wang *et al*., 2007). Sensitization to apomorphine (1.0 mg/kg) develops upon repeated administration, as assessed in an open field (Braga *et al*., 2009). This hyperactivity induced by apomorphine is suggested to be mediated by the stimulation of dopamine autoreceptors (Mattingly, Caudill and Abel, 2001). Withdrawal from repeated apomorphine (1.0 mg/kg) administration elicits reinforcement that could be monitored in a Conditioned Place Preference paradigm (Ma *et al*., 2006).

A possible reason for the compulsive use of apomorphine and other abused drugs could be the decrease in the activity of dopaminergic neurons following withdrawal (Haleem *et al*., 2005). A decrease in dopamine levels in the nucleus accumbens is reported to be associated with the symptoms of craving and drug withdrawal. Zijlstra *et al*., (2008) has reported that baseline availability of D_2 receptors is associated with cue-induced craving. Whereas, striatal dopamine release are associated with cue-elicited chronic cravings in opiate-dependent males. The acute use of cocaine also results in a brief and rapid increase in dopaminergic neurotransmission (Volkow *et al*., 2003). We measured not only the levels of neurotransmitters (biogenic amines; i.e., serotonin and dopamine) but also their metabolites in the brain samples. As neurotransmitter release in the synapse could be effectively measured by measuring their metabolites in the extracellular fluid to monitor neurotransmission process (Peters *et al*., 2000).

Antiparkinsonian action of apomorphine is qualitatively comparable to that of levodopa (Hagell and Odin, 2001). Though cocaine binds to several known receptors in brain, dopamine transporters are suggested to be particularly important in cocaine craving and self administration (Ritz *et al*., 1998; Kiyatkin *et al*., 2000). Lyness *et al*., (1979) have reported that the nerve endings of dopaminergic neurons present in nucleus accumbens are necessary for the acquisition as well as maintenance of self administration of d-amphetamine. Thus, dopamine is of prime importance in manifesting the craving for abused drugs. Volkow *et al*., (2009) have reported that the reinforcement by the abused drugs increase the release of dopamine.

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Apomorphine (2.0 mg/kg) induced locomotor sensitization varies with peak concentration of drug as well as the habituation. While studying sensitization effects of psychostimulants, it is important to consider post injection (Braga *et al*., 2009). Repeated apomorphine (1.0 mg/kg) administration increases behavioral sensitivity, which could be attenuated upon repeated coadministration with 7-hydroxy 7-hydroxy-N,N-dipropyl-2-aminotetralin (7-OH-DPAT; dopamine receptor agonist) (Mattingly *et al*., 2001). This apomorphine-induced sensitization could be monitored after single injection of the drug as well (Bloise, Carey, Carrera, 2001). Sensitivity to reinforcement has been reported to vary with various doses of apomorphine (Bratcher *et al*., 2005). Researchers have reported that serotonin and dopamine modulate the neurotransmission of each other (Zangen *et al*., 2001; Neumaier *et al*., 2008; Ikram, Samad and Haleem, 2007; Ikram and Haleem, 2010). Serotonin has an inhibitory effect dopaminergic neurotransmission while an increase in dopaminergic activity may also modulate serotonergic functions (Haleem *et al*., 2002). The present experiment was designed to monitor the dose-dependent effect of apomorphine on motor activity and monoamine metabolism.

MATERIALS AND METHODS

Animals

Experimental design was carried out in strict accordance with the guidelines by the Institutional Animal Ethics Committee (IAEC). Albino-Wistar rats (weighing 180-220 grams) provided by the HEJ Research Institute of Chemistry, University of Karachi were housed individually in perspex cages. Animals were placed in an environmentally controlled room at room temperature (25 ± 2°C) under a 12:12 h light/ dark cycle (lights on at 6:00 hr). A three day acquisition phase was allowed before starting the experiments so that the animals could become familiar with the environment.

Drug and doses

Apomorphine-HCl (Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intra-peritoneally at a dose of 1.0 mg/kg, 2.0 mg/kg and 4.0 mg/kg to the respective animals. Drug was freshly prepared before starting the experiment. Saline (0.9% NaCl solution; 1ml/kg) was injected to control animals.

Experimental protocol

Animals were randomly divided into four groups each containing six animals. These groups were labeled as: (i) saline injected, (ii) Apomorphine (1.0 mg/kg)-, (iii) Apomorphine (2.0 mg/kg)-, and (iv) Apomorphine (4.0 mg/kg) injected rats. Rats were injected with saline (0.9% NaCl solution) or respective dose of apomorphine. Motor activity in familiar environment (home cage) was monitored 5min post apomorphine injection. While exploratory activity in open field was monitored 15min and 40min post injection respectively. Animals were then decapitated 1hr post injections to collect brain samples. Samples were kept at -70 °C until neurochemical analysis by High Performance Liquid Chromatography (HPLC-EC).

Behavioral Procedures

Dose-dependent effect of apomorphine on motor activity in a familiar environment

15 min before injection, animals were transferred to the activity cages (transparent perspex cages with dimensions 26x26x26 cm) with saw dust covered floor. Activity was monitored as counts of cage crossings/10 min starting 5min post injections (Ikram, Samad and Haleem, 2007).

Dose-dependent effect of apomorphine on exploratory activity in a novel environment

15 and 40 min post injection, activity in open field was monitored. Procedure was same as described before (Ikram, Samad and Haleem, 2007). Activity was recorded as numbers of squares crossed with all four paws for 5 minutes.

Decapitation of rat brain

Saline or apomorphine (1.0, 2.0 and 4.0 mg/kg) injected animals were killed 1hr post injections. The 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. The collected brains were immediately stored at −70°C for the estimation of biogenic amines and metabolites using High performance liquid chromatography with electrochemical detection (HPLC-EC) (Ikram, Samad and Haleem, 2007).

HPLC-EC analysis of DA, 5-HT and metabolites

Extraction of biogenic amines and metabolites was same as described previously (Ikram, Samad and Haleem, 2007). Extraction was performed using 70% perchloric acid. 5 times volume of the extraction medium was added to the brain tissues. Samples were homogenized by using electrical homogenizer and subjected to ultra-centrifugation at 6000rpm for 20min at 4°C. Supernatant was separated and injected to HPLC-EC for neurochemical assay. HPLC-EC estimation was done as described earlier (Ikram and Haleem, 2010). A 5µ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. 0.1 M phosphate buffer (PH 2.9) containing EDTA (0.0035%), methanol (14%) and octyl sodium sulfate (0.023%) was used at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection (using Schimadzu LEC 6A detector) was done at an operating potential of +0.8V.

STATISTICAL ANALYSIS

All results are given as means ± S.D. Analysis of the data was performed by SPSS software (version 16.0) using one-way ANOVA (analysis of variance). Newman-Keuls test was used for *post hoc* comparisons. Results with *p* values p<0.05 were considered statistically significant.

RESULTS

Effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine on home cage activities are shown in figure 1. Effects of apomorphine were significant (F = 17.044; df = 1,20; $p<0.05$). Apomorphine increased activity at all three doses ($p<0.01$)as compared to the saline injected controls. At moderate and high doses (2.0 & 4.0 mg/kg respectively) of apomorphine, the increase in activity was comparable to low dose (1.0mg/kg) injected animals ($p<0.05$ & $p<0.01$ respectively).

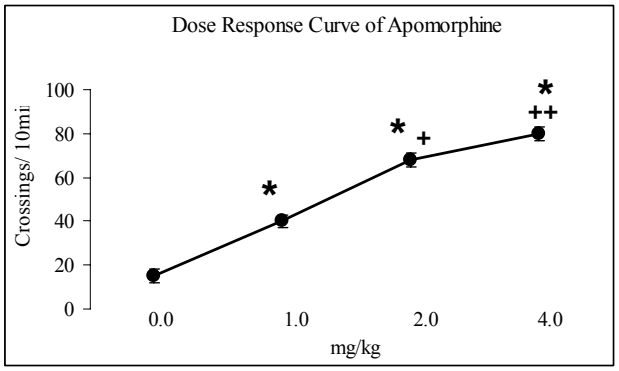

Fig. 1: Effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine administration on locomotor activity in a familiar environment. Values are means ± SD (n=6). Significant differences by Newman-keuls test: *$p<0.01$ from saline injected controls; +$p<0.05$, ++$p<0.01$ from apomorphine (1.0 mg/kg) injected rats following one-way ANOVA.

Figure 2 shows effects of different doses (1.0, 2.0 and 4.0 mg/kg) of apomorphine on exploratory activity in open field), 15 and 40 min post injection. Effect of apomorphine (F=152.70; df=1,40; $p<0.01$), repeated monitoring (F=59.41; df=3,40; $p<0.01$) and interaction between the two (F=294.26; df=3,40; $p<0.01$) were all significant. Administration of apomorphine increased activity in the familiar environment with an increase in drug dose. The increases in the activity at the doses of 1.0, 2.0 & 4.0mg/kg were significantly increased ($p<0.01$) from saline injected controls. The increases in the activity at the doses of 2.0 & 4.0mg/kg compared to 1.0mg/kg apomorphine injected rats were $p<0.05$ and $p<0.01$ respectively. The increases in the activity at the doses of 4.0 mg/kg as compared to 2.0 mg/kg were not significant.

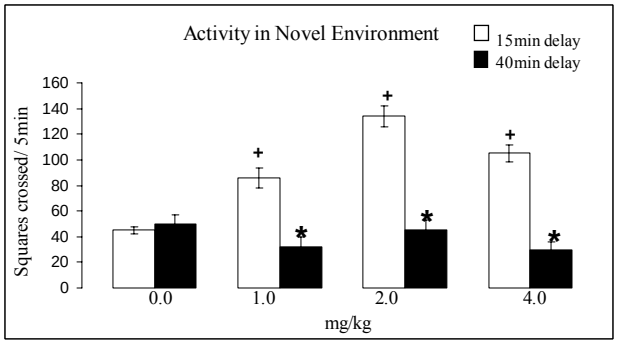

Fig. 2: Effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine administration on exploratory activity in a novel environment as monitored 15min and 40min post apomorphine injection. Values are means ± SD (n=6). Significant differences by Newman-keuls test: +$p<0.01$ from controls (0.0mg/kg); *$p<0.01$ from similarly treated animals (15min delay) following two-way ANOVA.

Figure 3 shows effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine on serotonin metabolism in whole brain samples. Apomorphine had no significant effect on 5-HT (F = 1.167; df = 1,20) as well as 5-HIAA (F = 1.057; df = 1,20) levels in the whole brain samples.

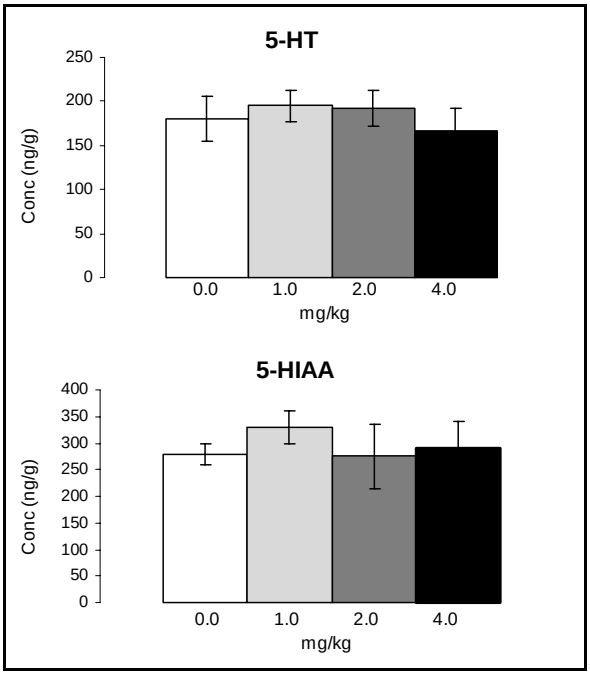

Fig. 3: Effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine on 5-HT and metabolite; 5-HIAA. Values are means ± SD (n=6). Differences between groups were not significant as analyzd by one-way ANOVA.

Figure 4 shows effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine on dopamine metabolism in whole brain samples. Significant effect of apomorphine on DOPAC (F = 7.91; df = 1,20; $p<0.01$) and HVA (F = 4.766; df = 1,20; $p<0.05$) levels were observed. However,

effect on DA (F = 1.679; df = 1,20) was not significant. Significant ($p<0.01$) decrease in DOPAC levels was observed in high dose (4.0 mg/kg) apomorphine injected rats, as compared to saline as well as low dose apomorphine injected rats (1.0 mg/kg). Apomorphine at moderate (2.0 mg/kg) dose increased ($p<0.05$) HVA levels as compared to saline injected controls. Whereas, at high dose (4.0 mg/kg) HVA levels were decreased ($p<0.01$) as compared to rats injected with moderate (2.0 mg/kg) dose of apomorphine.

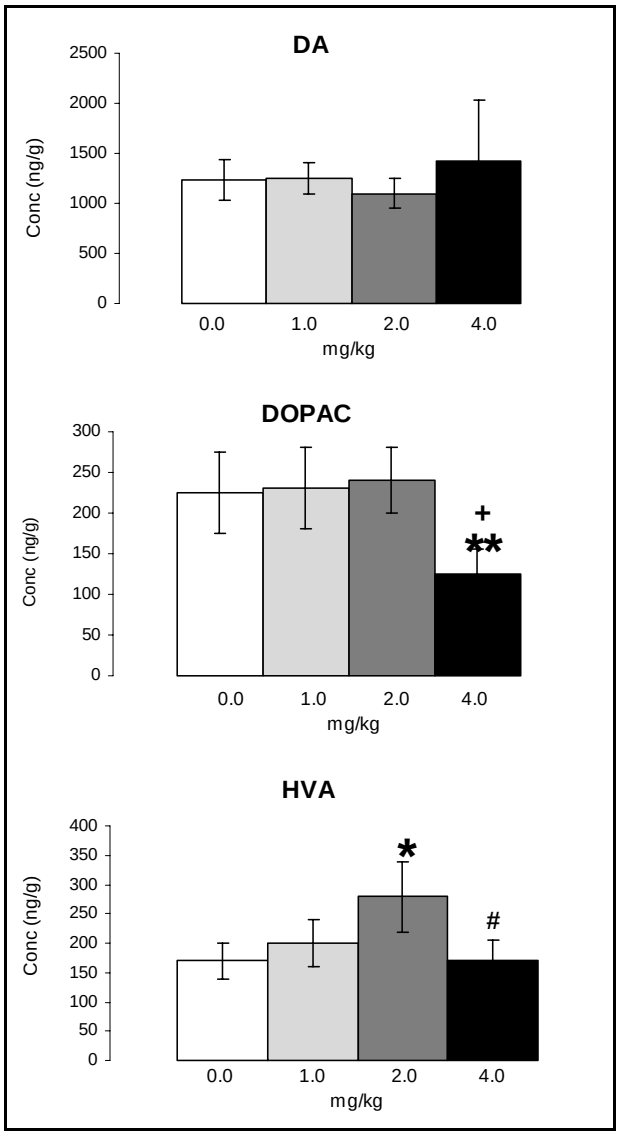

Fig. 4: Effects of different doses (1.0, 2.0 & 4.0 mg/kg) of apomorphine on dopamine and metabolites; DOPAC and HVA. Values are means ± SD (n=6). Significant differences by Newman-keuls test: *$p<0.05$, **$p<0.01$ from saline injected controls; +$p<0.01$ from apomorphine (1.0mg/kg) injected rats; #$p<0.05$ from apomorphine (2.0mg/kg) injected rats following one-way ANOVA.

DISCUSSION

In the present study, effects of apomorphine on locomotor activity and monoamine metabolism were observed. Since other authors have reported the development of context specific sensitization with CNS stimulants, as monitored in novel environment of an open field (Bloise, Carey and Carrera, 2007), we monitored the activity in familiar environment of home cage as well as novel environment of an open field apparatus, to compare the dose dependent effects. Since activity in the familiar environment was paired with drug administration (5min delay), apomorphine dose dependently increased the motor activity in familiar environment (fig. 1). In novel environment similar effects were observed when activities were monitored 15min post apomorphine injection (drug paired with test arena). However, monitoring the activities in novel environment 40min post injection, no significant effect of apomorphine was observed (fig. 2). Various researchers have reported different findings regarding the locomotor activity of animals in open filed. These varying effects were due to variation in the test arena as well as pretest habituation to the arena (Muller *et al*., 2007; De La Garza *et al*., 2000; Kuczenski *et al*,. 1999; Przegalinski *et al*., 2000). Therefore, we can conclude that the pairing of open field with drug administration is very important for monitoring the behavioral sensitization. If animals are exposed to the testing arena after considerable delay, they could associate it with the negative effects of drug (i.e., relapse, craving). Therefore, we monitored the time-dependent effect of single apomorphine injection at varying doses. Following our observations, it is suggested that the apomorphine-induced sensitization monitoring could not be done after 40min.

Results from the present study also suggest that apomorphine-induced sensitization effects could be monitored after single treatment, if evaluated in the test arena paired with drug administration. Previous papers from our laboratory have reported the development of apomorphine-induced sensitization following its repeated administration in familiar but not in novel environment (Hasnat and Haleem, 2005; Haleem *et al*., 2005). Braga *et al*. (2009) have suggested that acute administration of cocaine increases activity during early testing session (around 15min post injection) whereas chronic (12 days) administration results in a delayed onset of hyperactivity (Ansah, Wade and Shockley, 1996; Post and Rose, 1976).

Dopamine is of prime importance in manifesting the reinforcing effects of psychostimulants. All drugs of abuse increase the release of dopamine in nucleus accumbens irrespective of their initial target (Pierce and Kumaresan, 2006; Vetulani, 2001). Administration of D_2 antagonist (such as sulpiride) into the nucleus accumbens dose-dependently can attenuate cocaine reinstatement (Anderson and Schmidt, Pierce, 2006). Antagonism of

apomorphine-induced effects by sulpiride has also been reported by Puech, Simon and Boissier (1976).

5-HT is also involved in the development of behavioral sensitization by repeated administration of apomorphine and other psychostimulants (Ikram and Haleem, 2011). Loss of 5-HT gene can alter behavioral as well as neurochemical effects of ethanol and 5-HT knockout mice exhibit increased sensitivity to sedation/hypnosis induced by ethanol (Boyce-Rustay *et al*., 2006). We did not monitor any significant alteration in 5-HT metabolism following single apomorphine injection at various doses (Figure 3). This could be due to the reason that apomorphine-induced 5-HT alterations would not have been there after 60min post injection. It is therefore recommended that in future, brain samples should be collected before 40min post injection, so as to monitor the effects produced by drug. Since activities in home cage and open field were altered before 40min, we recommend that the alterations in 5-HT metabolism would have been there but these alterations may not have been substantial enough to maintain themselves over longer period of time.

In addicts, dopaminergic functions are decreased upon drug withdrawal, which also reduces their response to natural reinforcers, resulting in their lack of pleasure and interest in routine-activities/tasks. Therapeutic approaches include motivation of these addicts so that they could be engaged in non-drug related behaviors (Volkow *et al*., 2009). It is also suggested that presentation of cues (to addicted animals) associated with drug use, can also activate dopaminergic system. Environment can also profoundly affect drug abuse relapse, maintenance and vulnerability (Nader and Czoty, 2005; Panlilio *et al*., 2005). In the present study, increased levels of HVA, a metabolite of dopamine, was monitored in the rat brain following apomorphine injections at a dose of 2.0 mg/kg (Fig 4). Increased DA metabolism, particularly in the nucleus accumbens, could be well-associated with the motivation upon apomorphine administration. An increase in DOPAMINE metabolism is also well associated with behavioral sensitization (Shim *et al*., 2001). We monitored increase in activity of animals in home cage as well as open field but complementary changes in the dopamine or its metabolites were not observed due to the reason that apomorphine-induced alterations in levels of dopamine and metabolites would not have been there after 60min post injection.

At the dose of 4.0 mg/kg, apomorphine decreased ($p<0.01$) HVA and DOPAC levels (Fig. 4). This decrease in DOPAC and HVA levels could be due to the reason that we collected brain samples 1hr post apomorphine injection. Half-life of apomorphine is about 27-31 minutes (Symes *et al*., 1976; Sam *et al*., 1995) which suggests that after 30-40 minutes apomorphine levels would decline in circulation. We observed decreased metabolism of dopamine as we decapitated animals 60min post injection. This decreased dopamine metabolism (60min post injection) could well be associated with the withdrawal of apomorphine. This decreased dopamine metabolism particularly in the caudate, may well be associated with the withdrawal symptoms for the abused drug (Espejo *et al*., 2001).

CONCLUSION

From results of the present study we can conclude that administration of apomorphine increased dopamine metabolism at moderate dose (2.0 mg/kg). Whereas, decreased metabolism of dopamine was observed following administration of the drug at 4.0 mg/kg. This suggests that like the reinforcing effects, withdrawal from drug administration is more pronounced at high doses. Apomorphine increased motor activity in the novel and familiar environment in a dose-dependent manner, provided that the drug was paired with the testing environment. Since results from both familiar and novel environments were parallel, any of these could be used for studying the sensitization effects of apomorphine. Familiar environment should be preferred for monitoring sensitization effects as the daily exposure of animals to a novel environment could result in familiarization. However, the effects of apomorphine in a novel environment could be monitored before and after the treatment session to observe its effects on motor activity. It would be imperative to monitor context-specific motor activity upon repeated administration of apomorphine in future studies.

ACKNOWLEDGEMENT

Authors would like to thank Higher Education Commission for the research grant.

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